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Association between the Number of Chondrocytes of Lumbar Intervertebral Discs and Age, Abdominal Aorta Atherosclerosis and Lumbar Artery Arteriosclerosis "A Descriptive Cross Sectional Study"

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Abstract

Background: The effect of age, atherosclerotic changes of abdominal aorta and arteriosclerotic changes of lumbar arteries on number of chondrocytes of lumbar intervertebral discs has not been studied.

Methods: A descriptive cross sectional post mortem study was carried out in Faculty of Medicine, University of Kelaniya, Sri Lanka to determine the association between the mean number of chondrocytes in the fifth lumbar intervertebral disc and age, the level of atherosclerotic changes of the lumbar abdominal aorta and arteriosclerotic changes of right and left, fourth lumbar arteries. The presence or absence of atherosclerotic plaques in the abdominal aorta lumbar region was studied. To assess arteriosclerotic changes, the Intima Media thickness (IMT) and the total thickness (TT) of lumbar arteries were measured and the ratio IMT/TT was calculated. Bivariant analysis and a multi variant analysis were done.

Results: There were 51 samples in total. Their ages ranged from 18 to 96 years. The mean age (SD) was 43 (± 17.1) years. The samples were obtained from 38 males and 13 females. There was a significant negative association ($P<0.001$; Beta Coefficient=- 0.95) between the age of the subject and the mean number of chondrocytes in the intervertebral disc. There was no significant association between atherosclerotic changes of abdominal aorta and arteriosclerotic changes of lumbar arteries and the mean number of chondrocytes.

Conclusion: Only the age had a significant negative association with the mean number of chondrocytes of the intervertebral disc.

Keywords: Atherosclerosis; Intervertebral disc; Abdominal aorta; Chondrocytes

Introduction

It is estimated that in every population, an individual has an 80% probability of having low back pain at some period during their life time [1]. Low back pain is defined as a pain or discomfort in the lumbar sacral region [2]. Low back pain and lumbar sacral radicular pain have a large number of causes and out of those causes; intervertebral disc degeneration is one of the most common causes [3]. Intervertebral disc degeneration is an important cause of persistent low back pain with or without leg pain [4].

The intervertebral disc is a soft tissue, positioned between each of the vertebrae to accommodate applied biomechanical forces to the spine. The central compartment of the disc contains the gelatinous nucleus pulposus, which is enclosed by the tough annulus fibrosus and the endplate cartilage [5]. The nucleus pulposus is highly pressurized and the annulus fibrosus prevents radial disc bulge which resists large tensile and compressive strains. The cartilaginous endplate is an interface tissue connecting the disc to the adjacent vertebral bodies, and functions to distribute stresses between the disc and the vertebrae and to act as a gateway for nutritional transport in the avascular disc [6]. Chondrocytes are the dominant cell type found in the disc and these cells are responsible for the synthesis, maintenance, and gradual turnover of the extracellular matrix. The cartilage extracellular matrix is remodeled in response to the functional demands of mechanical loading, and this process is mediated through the metabolic activity of chondrocytes [7]. Any structural defect and/or metabolic disturbances in the extracellular matrix may cause cellular and tissue alterations that can lead to the development or progression of disc degeneration [8]. Degeneration of the intervertebral disc is a process characterized by a cascade of cellular, biochemical, structural and functional changes of the disc. A key factor in early disc degeneration is the decrease in proteoglycan content. [9]. During intervertebral disc degeneration (IVD), chondrocytes respond to early degeneration by increasing their biosynthetic processes [6]. Certain scientists believe that re-implantation of in vitro-activated autologous chondrocytes may be useful in reversing the disc degeneration process [10].

In aged tissues, the extra cellular material is not efficiently maintained and it is believed that chondrocytes may exhibit altered mechano-responsive properties as they age. Another change observed with regards to age is that the stiffness of chondrocytes increases with advancing age [7]. In the above studies, what happens in regards to the number of chondrocytes with an increase in age has not been mentioned.

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In adult populations, atherosclerosis has been found to be associated with disc degeneration [11]. This study has found an association between atherosclerosis of abdominal aorta and macroscopic changes in the intervertebral discs. Studies done by Suri et al. found that the increase in abdominal aortic calcification had an association with reduction in lumbar disc height [12]. However, both of these studies do not mention about the microscopic changes that occur in the intervertebral discs and its cells with atherosclerotic changes of the abdominal aorta. According to studies done by Carr et al. (2008) there was a negative association between calcified atheromatous plaques of abdominal aorta and bone mineral density of thoracic and lumbar spines [13]. This study demonstrates the effect of atherosclerosis of abdominal aorta on thoracic and lumbar vertebral bodies but does not mention any effect on the thoracic and lumbar intervertebral discs that are important in providing support to the spine.

Arteriosclerosis literally means "hardening of the arteries"; it is a generic term reflecting arterial wall thickening and loss of elasticity. Arteriolosclerosis affects small arteries and arterioles, and may cause ischemic injury to tissues [14]. The predominant cellular element of the tunica media is smooth muscle cells. They have the capacity to proliferate when appropriately stimulated and can synthesize extracellular collagen, elastin, and proteoglycans and elaborate growth factors and cytokines. Smooth muscle cells are also responsible for the vasoconstriction or dilation that occurs in response to physiologic or pharmacologic stimuli [14].

It is known that arteries lose their compliance with advancing age even in the absence of concurrent cardiovascular disease. In most studies age has been shown to have a predominant effect on indices of arterial mechanical behavior. It is also apparent that different arterial segments respond differently to aging, probably related to differences in elastin-collagen smooth-muscle proportions. Information regarding arterial patho-physiology may be obtained by comparing age-related changes in the different arterial segments in normal and in subjects with disease [15]. Lumbar arteries are muscular arteries that supply intervertebral discs in the lumbar region. Age related changes of lumbar arteries have not been studied. Increased intima-media thickness (IMT) is a marker of arterial wall alteration. It can result from cardiovascular risk factors such as smoking, hypertension, diabetes mellitus, and hypercholesterolemia. However it may not necessarily reflect pathologic changing because it is closely correlated to age. Increased IMT of arteries is directly associated with an increased risk of cardiovascular disease and its measurement is well accepted for clinical research [16].

Studies done with regards to changes that occur in the intervertebral disc due to atherosclerotic changes of abdominal aorta and arterio-sclerotic changes of lumbar arteries are sparse and there are no studies that describe the association between the atherosclerotic changes of the abdominal aorta and arterio-sclerotic changes of lumbar arteries and the number of chondrocytes in the intervertebral discs. Chondrocytes play a key role in maintaining the structure of the intervertebral discs and alterations in the function of chondrocytes can play a significant role in disc degeneration. Although some studies mention that degenerative changes of the disc increases with advancing age and chondrocytes play a vital role in maintaining the structure of the disc, there are no studies that have been done to find out the association between the number of chondrocytes in the disc and the age of the subject. Therefore this study was carried out to determine the association between the mean number of chondrocytes in the intervertebral disc and the age of the subject, atherosclerotic changes of the lumbar abdominal aorta and the arteriosclerotic changes of lumbar arteries and to determine the association between the age and atherosclerotic changes of the lumbar abdominal aorta and the arteriosclerotic changes of lumbar arteries.

Materials and Methods

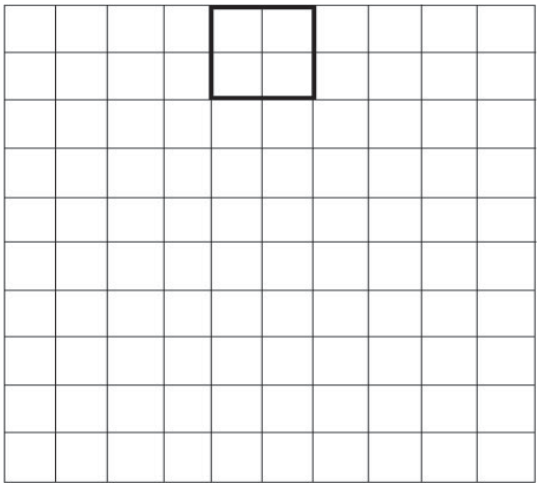
A descriptive cross-sectional study was done in Faculty of Medicine, University of Kelaniya, Sri Lanka. The ethical clearance for the study was obtained by the ethical review committee of Faculty of Medicine, University of Kelaniya, Sri Lanka. The tissues from the postmortem bodies were collected with the consent of either of the relatives (mother, father, brother, sister, son and daughter).

Postmortem samples of fifth lumbar intervertebral discs and lumbar part of the abdominal aorta up to the bifurcation of the aorta was taken from each body at the postmortem. The fifth lumbar intervertebral disc was selected because it bears the highest amount of weight. The tissues from the postmortem bodies were collected within twelve hours of death. After removal of the whole fifth lumbar inter vertebral disc from the body, a pyramidal shaped piece (2 cm at the base, 0.5 cm in the direction of apex and 0.3 cm thick) was cut from the anterior part of the annulus fibrosus of the disc. The piece of tissue was cut in a way that the base of the pyramid was at the periphery of the disc. It was fixed in 10% formal saline in a properly labeled container.

A 2 mm length section (from the attachment of abdominal aorta) was also taken from left and right fourth lumbar arteries and fixed in 10% formal saline.

After processing of the tissues from intervertebral discs and lumbar arteries according to guidelines given by Anderson and Bancrofti [17]. Haematoxylene and eosin stained slides were prepared according to the guidelines given by Wilson and Gamble [18].

A light microscope (Olympus) with a vernier scale in the x-axis and y-axis was used for counting of chondrocytes. In addition, a graticule was connected to the microscope. The graticule had a square shaped counting surface area of 100 mm^2. This area had 100 of 1 mm^2 squares (Figure 1). The two squares in the 5^{th} and 6^{th} columns in the first row and two squares in the 5^{th} and 6^{th} columns in the second row were used for counting chondrocytes (Figure 1). Out of the upper rows of the graticule these squares were the most clearly visible



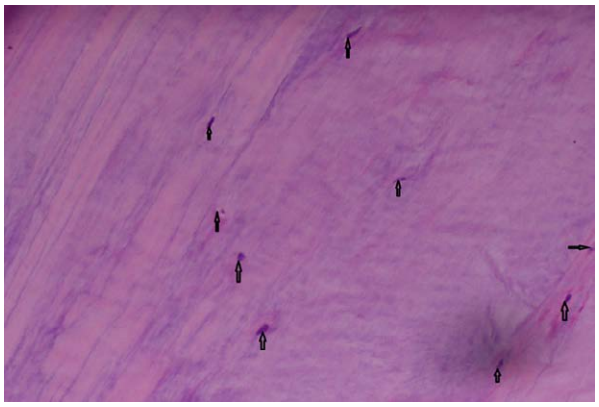
Squares in highlighted area were used for counting of chondrocytes.

Figure 1: The graticule that was used for counting of chondrocytes.

under the microscope and therefore they were used for counting of chondrocytes. Annulus fibrosus area was used for cell counting because the chondrocytes were commonly present in the annulus fibrosus. For cell counting, high magnification (10 × 40) was used. During counting, the cells touching the outer four borders of the counting area were excluded. The cells touching the inner vertical line belonged to the 5th column and cells touching the inner horizontal line belonged to the second row. The counting begun at the upper left hand corner of the section and was moved vertically downwards along each section till chondrocytes were found [19]. At the end of the vertical direction of the section, the section was moved in the horizontal direction. Each time the section was moved, the readings in the vertical and horizontal scales of the microscope were recorded and each movement was numbered. This was important in reproducing the results. Ten high-power fields were counted and the average was taken [19]. The cells were counted by a consultant pathologist. To check the reproducibility of results, ten histological sections were counted by two consultant pathologists. The differences between the mean numbers of cells counted by the two pathologists in all the ten sections were insignificant. A cell located in a lacuna was identified as a chondrocyte and if there were more than one cell in a lacuna they were counted as separate cells (Figure 2).

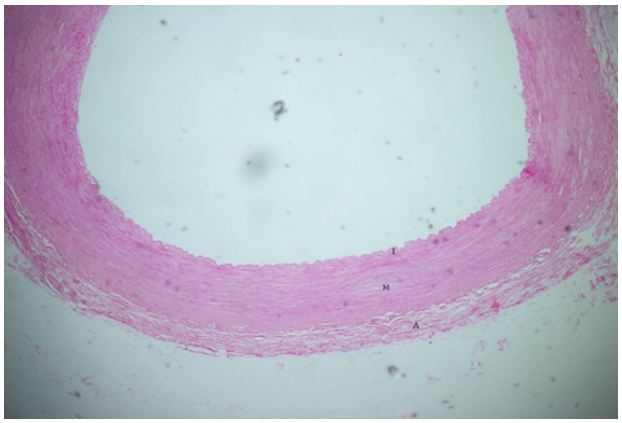
After preparing cross section slides of lumbar arteries the intima media thickness (IMT) and total thickness (TT) of lumbar arteries were measured using the vernier scales in the light microscope (Olympus). These measurements were done while the slides were viewed under the high power of the microscope (Figure 3). The length from the lumen end of tunica intima to the outer end of smooth muscle cell layer of the tunica media was considered as the IMT. Due to difficulty in differentiating the outer layers of tunica media and the inner layer of tunica adventitia the outer end of the smooth muscle cell layer of tunica media was considered as the outer end of the tunica media. The length from the lumen end of tunica intima to outer end of the tunica adventitia was considered as the TT. Thereafter the IMT/TT ratio was calculated. This ratio was calculated to assess the severity of arteriosclerosis of lumbar arteries

The inner surface of the lumbar region of abdominal aorta was exposed to study the severity of atherosclerosis. The severity of atherosclerosis was assessed according to the guidelines given in American heart association classification of human atherosclerotic lesions [20]. The specimens which showed no evidence of atheromatous



Arrows indicates chondrocytes. There are nine chondrocytes.

Figure 2: Annulus fibrosus of intervertebral disc with chondrocytes in a high power field (10 x 40).



I – Tunica intima, M – Tunica media, A – Tunica adventitia

Figure 3: Tunica intima, media and adventitia of lumbar arteries viewed under high power field (10 x 40).

plaque formation were graded as grade1, where there was only simple atheromatous plaque formation were graded as grade 2 and specimens which showed evidence of ulcerated calcified atheromatous plaques were graded as grade 3. To find out the association between the mean number of chondrocytes in the intervertebral disc and the levels of atherosclerosis of abdominal aorta and levels of arteriosclerosis of lumbar arteries the mean number of cells less than $3/mm^2$ and $>3/mm^2$ was used to divide the study sample into two groups. The results were analyzed using the SPSS version 21 Statistical package. Bivariant and multivariate analysis both were done. Odds ratio was done to calculate the strength of association.

Results

There were 51 samples in total. Out of which, 38 samples were obtained from males and 13 samples were obtained from females. The study subjects' ages ranged from 21 to 96 years. The mean age was 43 years and the standard deviation was ± 17.1 years.

The mean (SD) number of chondrocytes in males were 3.4 (1.2) and the mean (SD) number of chondrocytes in females were 3.8 (0.2). The mean (SD) age of males were 50 (18.9) years and the mean (SD) age of females were 33 (3.4) years (Table 1).

Atheromatous changes were present in 65% of specimens. In 35% of specimens, there was no evidence of atheroma formation. They were people from the range of ages 21-45 years and their mean age (SD) was 31.25 years. All the specimens obtained from individuals over 45 years of age showed evidence of atheroma formation in the lumbar abdominal aorta. Grade 3 (severe atheromatous) changes were observed in all the people who were older than 50 years. There was a significant ($P \leq 0.001$) association between atheromatous changes of abdominal aorta and the age of the subjects (Table 2). The association between the age of the subjects and IMT/TT of left lumbar artery ($r=0.903$, $P \leq 0.001$) and IMT/TT of right lumbar artery ($r=0.886$, $P=<0.001$) was also significant (Table 2).

According to the results of bivariant analysis the age ($r=-0.827$, $P \leq 0.001$), levels of atherosclerosis ($r=-0.568$, $P \leq 0.001$), IMT/TT of left lumbar artery ($r=-0.721$, $P \leq 0.001$) and IMT/TT ($r=-0.737$, $P \leq 0.001$) of right lumbar artery had a significant negative association with the mean number of chondrocytes (Table 3).

	Males (Number=38)	Females (Number=13)
Mean (SD) Age	50 (18.9)	33 (3.4)
Mean (SD) number of Cells	3.8 (0.2)	3.4 (1.2)

Table 1: The comparison between males and females with regard to the Mean number of chondrocytes.

Variable	Pearson correlation	Level of significance (P value)
Atherosclerosis	0.748	<0.001
Left lumbar artery IMT/TT	0.903	<0.001
Right lumbar artery IMT/TT	0.886	<0.001

Table 2: Results of Bivariant analysis demonstrating the association between the age of the subject and atheromatous changes of the abdominal aorta and IMT/TT ratio of left and right lumbar arteries.

Variable	Pearson correlation	Level of significance (P value)
Age	- 0.827	<0.001
Atherosclerosis	- 0.568	<0.001
Left lumbar artery IMT/TT	- 0.721	<0.001
Right lumbar artery IMT/TT	- 0.737	<0.001

Table 3: Results of Bivariant analysis demonstrating the association between the age, atheromatous changes of the abdominal aorta and IMT/TT ratio of left and right lumbar arteries on mean number of disc chondrocytes.

Variable	Beta coefficient	Level of significance (P value)	95% Confidence Interval	
			Lower	Upper
Age	-0.949	<0.001	-0.071	-0.03
Atherosclerosis	0.068	0.600	-0.248	-1 .425
Left lumbar artery IMT/TT	0.479	0.197	-1.869	-8.836
Right lumbar artery IMT/TT	0.409	0. 220	-7.945	1.876

Table 4: Results of Multi variant analysis demonstrating the association between the age, atheromatous changes of the abdominal aorta and IMT/TT ratio of lumbar arteries on the mean number of disc chondrocytes.

According to the results of multivariate analysis only the age had a significant negative association with the mean number of chondrocytes (Beta coefficient=-0.949, P ≤ 0.001). Atheromatous changes of abdominal aorta and IMT/TT of left and right lumbar arteries did not have a significant association with the mean number of chondrocytes (Table 4).

Discussion

This study is one of the very few studies that have been done to demonstrate an association between the mean number of chondrocytes in the annulus fibrosus of lumbar intervertebral discs and age, atherosclerotic changes of abdominal aorta and arteriosclerotic changes of lumbar arteries.

Association between the age of the subject and the atheromatous changes of the lumbar abdominal aorta

In the current study of Sri Lankans, all the postmortem specimens collected from people who are over the age of 45 years showed evidence of atheroma formation in the vessel wall. According to studies done by Wilson et al., atherosclerotic plaques and calcified plaques were present in all the subjects after the age of 30 years [21]. Formation of atherosclerotic lesions can vary according to environmental and genetic factors [21]. These risk factors can vary between different populations. Previous studies have stated that atheromatous changes of the vessel wall increases with age of the subject and there are different types of atheromatous changes. In Sri Lanka, not many studies have been done to find out the ages at which these atheromatous changes are prevalent. According to the results of the present study grade 2, atheromatous changes were present from the ages ranging from 31 to 50 years and grade 3 atheromatous changes were present from the ages ranging from 37–96 years. According to the results of the present study age had a significant association (P ≤ 0.001) with the atheromatous changes of the abdominal aorta. With increase age, a variety of changes occur in the collagen and elastin fibers in the vessel wall [22]. A change in structural properties of the vascular wall, which leads to the loss of arterial elasticity, reduces the arterial compliance [23]. According to them, these changes cause an increased stress on the luminal wall thus increasing the possibility of endothelial damage and resulting in predisposition to atherosclerosis. This may be one of the reasons why in the present study, atheromatous changes of arteries had a significant association with the age of the subject.

Association between the age of subject and the arterio sclerotic (IMT/TT ratio) changes of lumbar arteries.

To measure the arterio sclerotic changes of lumbar arteries the IMT/TT ratio was used. According to our study the age had a significant positive association with the IMT/TT ratio of left (r=0.903, P ≤ 0.001) and right (r=0.737, P ≤ 0.001) lumbar arteries (Table 1). With increase in age there is proliferation of smooth muscle cells in the tunica media and increase in extracellular material in the tunica media [24]. Therefore this could be a reason why the IMT/TT ratio increased with the age. There were no studies describing the age related changes of lumbar arteries. Therefore our study is one of the first studies that have described one of the age related changes of lumbar arteries.

Association between the mean number of chondrocytes in the annulus fibrosus of the fifth lumbar disc and the age of the subject, atherosclerotic changes of lumbar abdominal aorta and arterio sclerotic changes (IMT/TT ratio) of left and right lumbar arteries.

According to the results of the current study, only the age of the subject had a significant negative correlation (r=-0.949, P<0.001) with the mean number of chondrocytes of intervertebral discs and there was no significant association between the mean number of chondrocytes and atherosclerotic changes of lumbar abdominal aorta and IMT/TT ratio of left and right lumbar arteries (Table 3). In the present study age had a significant association with atherosclerotic changes of abdominal aorta (P ≤ 0.001) and the arterio sclerotic changes (IMT/TT ratio) of left (P ≤ 0.001) and right (P ≤ 0.001) lumbar arteries (Table 1). Therefore this could be a reason why atherosclerotic changes of abdominal aorta and arterio sclerotic changes of lumbar arteries had a significant association with the mean number of chondrocytes of the intervertebral discs in the results of bivariant analysis but lost the significant association in the results of multivariate analysis.

Chondrocytes in the disc have many functions such as synthesizing and maintaining the matrices of intervertebral discs [7]. According to Smith et al. disc degeneration increases with age [9]. Takatalo et al. demonstrates that intervertebral disc degeneration increases with advanced atherosclerotic manifestations in the abdominal aorta [11]. Although certain studies state that disc degeneration increases with age and advanced atherosclerotic manifestations of abdominal aorta and chondrocytes of intervertebral discs are important for the maintenance of structure of the intervertebral discs, no studies have been done to find out an association between the number of chondrocytes in the

intervertebral disc and age, atherosclerotic changes of the abdominal aorta and arterio sclerotic changes of lumbar arteries.

Conclusions

In the present study atherosclerotic changes of abdominal aorta and arteriosclerotic changes of lumbar arteries did not have a significant association with the mean number of chondrocytes of the intervertebral disc. Only the age of the subject had a significant negative association with the mean number of chondrocytes of the intervertebral disc. In this study after the age of 45 years, all the subjects had atheromatous changes of the abdominal aorta and after the age of fifty 50 years all the subjects had severe atheromatous changes.

Our article provides information that may be useful to scientists that are investigating novel preventive or therapeutic options in intervertebral disc degeneration.

Limitations in this study

The present study was done on Heamatoxylin and Eosin stained slides using a light microscope. Therefore exact measurements of tunica intima, tunica media and tunica adventitia could not be obtained. Since a ratio of IMT/TT was used to compare the association with the mean number of chondrocytes it is unlikely that this limitation had an effect on the results of the study.

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