The Dynamics of the Vascular Wall Changes Evolution in the Lumbar Intervertebral Disc. Insights.

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***Abstract* — Atherosclerosis is one of the etiological factors to initiate degeneration disc studied in detail by histological investigations stained with haematoxylin-eosin and immunohistochemical examination with antibodies CD34, in immunohistochemical laboratory from Timisoara, leader Marius Raica. Were examined of 20 sample harversted from the L1-L5 vertebral arteries from cadavers with different cardiac pathologies as a couse of a death, age and sex from "Sfînta Treime" hospital. Atherosclerosis with vascular wall thickening affects the structureand viability of intervertebral disc, initially being affected nucleus pulposus, annulus fibrosis and then through a series of mechanisms it splits fragmented collagen fibers, and finally fibroblast proliferation with sclerosis. In five cases the vertebral arteries were affected, lacking intimate vascular event (5%), in three cases, faint, moderate; in ten cases-handed (50%), and in 6 cases-preserved.**

Currently modern technologies remain in the assessment of new methods of treatment and prevention of degenerative-dystrophic changes.

I. INTRODUCTION

The vertebral column diseases, associated with the degenerationive disorders in its structures, is one of the most actual problems in modern orthopedics. The prevention and treatment of these diseases requires a much larger research in the study of pathogenesis, depth and degree of involvement in the pathological process of spinal segments elements. Currently, it was found that the characteristic changes of pulposus nucleus are at the base of degenerative changes in the vertebral column, the mechanisms are not yet completely understood [1].

Based on the available information from literature [1], we can assume that among the factors contributing to the development of degenerative-dystrophic processes in tissues, the circulatory disorders of the spine have a substantial importance. However, so far there are any satisfactory answers to the questions relating to the initiation process of sclerosis and the early stages of development.

The sequence of degenerative disorders in the intervertebral disc is generally known only from the clinical observations, but a detailed evaluation of morphological and functional changes in the dynamics, by the modeling of pathological disorders, is possible only in an experimental study in animals. Some scientists have found a similarity in structural architecture of human and rabbit lumbar intervertebral disc [2]. In addition, a general nature of degeneration process in humans and rabbits was confirmed biochemically [3]. Especially, the nucleus differs in rodents and mammals, they are structured with the notochordal cells throughout life, while these cells disappear from the pulposus nucleus in humans after childhood [4].

During the ontogenesis the vascularization of disc undergoes some significant changes. By 25-26 years, the disc vascularization is provided to six arterial branches: each two dorsal blood vessels, the axial and ventral vessels. The arterial branches create the connections called "arcade". The vessels penetrate the discs through the vertebral bodies [5]. The vascular plexus are more dense on the front surface of disc [6]. In the end of body growth period from 25-26 years the disc vessels are coplect obliterated, the vascularization is provided to the diffusion from hyaline plate. Obermuth H., 1930, Coventry M., 1945 consider that that the disc is avascular and wear in the elderly persons. In the wear site is outlined a vascular proliferation with revascularization, which belongs to a pathological phenomenon.

Atherosclerosis [7,8], drepanocytic anaemia, Gaucher disease [9] are the factors that affect the blood irrigation in intervertebral discs. All lead to the disc degeneration in the long term with the circulation disorder in disc and surrounding tissues [10, 11]..

II. THE PURPOSE OF STUDY

The evalution of the degree of degenerative-dystrophic changes in the lumbar intervertebral disc by endothelial markers CD34 immunohistochemical staining of the lumbar vertebral arteries from the died patients of the different ages.

III. MATERIAL AND METHODS

The present paper is based on the study of the structure of vertebral lumbar artery and intervertebral disc by the

histological examination, hematoxylin-eosin staining (made in Moldova) and immunohistochemical staining with the antibodies CD34 (it determines the state of vascular intima and stem cells) from 2011-2012, being performed in Immunohistochemical Laboratory, Timisoara, Romania, under the leadership of Professor Marius Raica. The lumbar segments and the arteries were taken from 20 patients after cerebrovascular accidents, from acute cerebral infarct 10 persons (50%), hemorrhagic stroke 5 person (25%), diabetes mellitus type- 1 person (5%), acute myocardial infarct - 3persons (15%), hepatic cirrhosis-1 person (5%), of different age and sex, Clinical Hospital "Holy Trinity". The age of deaths in the study group: by 60 – 4 persons , 61-70 years -2 persons, 71-80 years - 8 persons, 81-90 years -6 persons.

The lumbar vertebral arteries were taken from each individual case beginning from the L1 until L5 levels and the L1 and L5 intervertebral discs.

Vascular wall atherosclerosis with the lipid spots deposition, the atherosclerotic plaques and the ulcerations are found in the section of abdominal aorta and iliac artery bifurcation [12, 13]. However, it is paradoxically that ateroslerotic plates deposition (Fig. 1) is more evident at the edges of inlets of lumbar vertebral paraaortic arteries I-IV (a) and V - by sacral average artery with the narrowing of lumen (b).

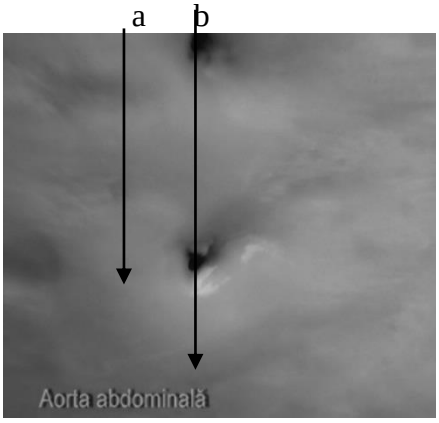

Figure 1. A 60-year-old patient J. The abdominal aorta: the inlets of narrowed vertebral artery L5 (a), surrounded by atherosclerotic plaques (b) macroscopic – the abdominal aorta with lipid spots and atherosclerotic plaque.

The immunohistochemical staining with endothelial marker CD34 shows the integrity of artery vascular intima in L1-L5 lumbar vertebral bodies taken from the death, the results are divided into four groups: IV- without intima (Fig. 2) where hematoxelină-eosin stained vertebral disc shows the advanced forms of intervertebral disc degeneration (Fig. 3) with disc cartilage hyalinisation (a), the intradiscal calcification or ossification areas (b), the lymphocyte infiltration (c).

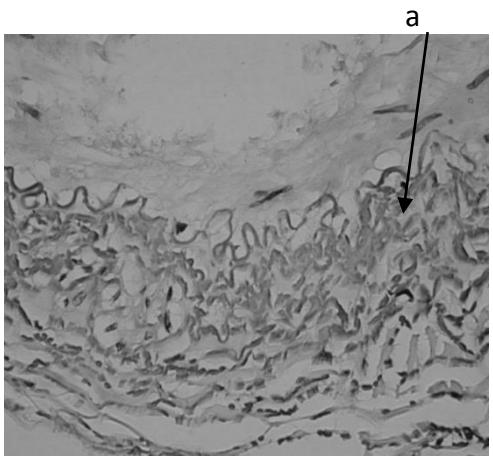

Fig. 2. 82-year-old patient K. The lumbar vertebral artery 5 a) the absence of basal membrane. CD34 IHC staining, x140

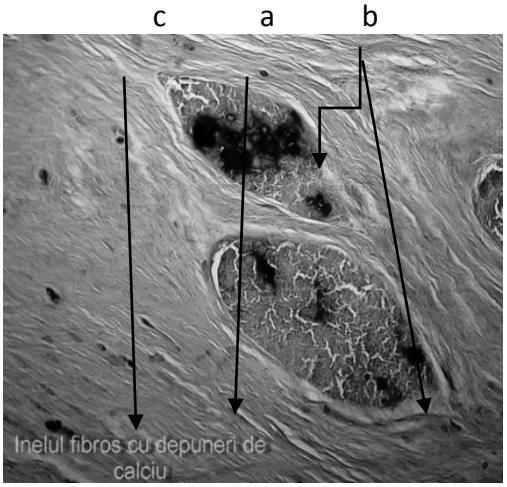

Figure 3. the same patients 82-year-old patient K. The fibers of intervertebral disc 5) The islets of hyalinic cartilage in the intervertebral disc, B) the intradiscal calcifications or ossification areas, c) the lymphocytic infiltration, HE staining, x 140.

The III group shows weak intima, for the example (Fig. 4) (a) the picture of the vertebral artery 4, where are shown well the internal tunic with endothelial cell layer (a), in half free, sometimes with a detachment, the average tunic with rare and disorganized fibrous elements in muscular layer (b) with thewell structured adventitia (c) the average forms of degeneration in intervertebral disc (Fig. 5), it is shown: a) the disc tissue fibrosis, with b) the areas of edema between the fibers, c) the disc cartilage with the chondrocytes into chondroblasts, d) and the papillary aspect e) the hemorrhagic areas, f) the hyalinisation areas, g) the fibroblast proliferation.

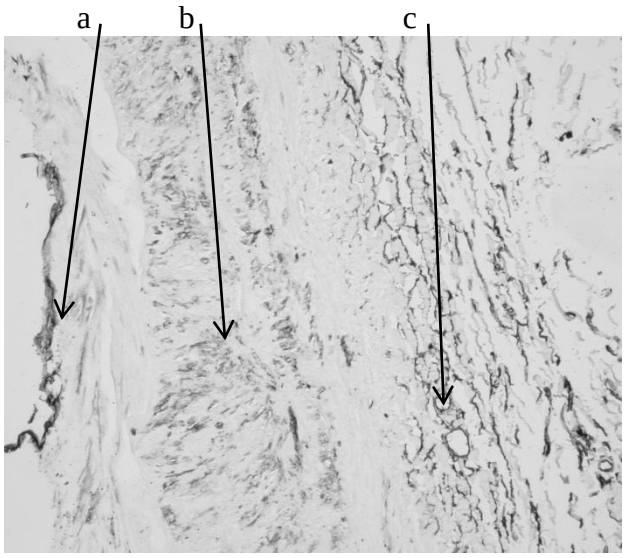

Figure 4. 62-year-old patient A. The lumbar vertebral artery 5 a) the presence of the half of basal membrane, with detachment, b) the average tunic with rare muscle fiber elements, c) the capillaries from

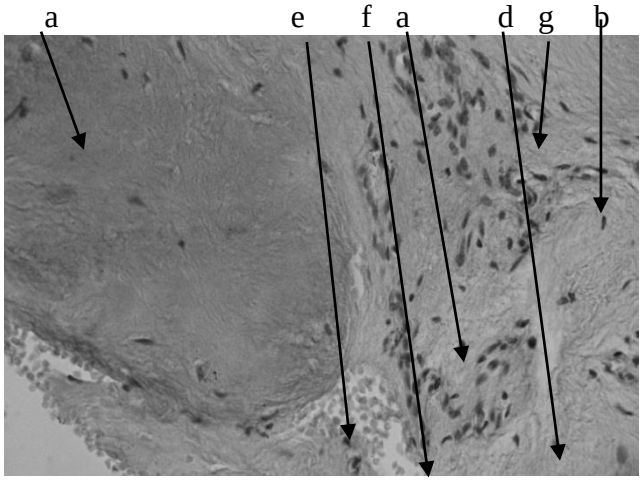

Figure 5. A 62-year-old patient A.The pulposus nucleus of lumbar intervertebral disc 4 5a) the disc tissue fibrosis, b) the areas of edema between fibers, c) the disc cartilage with chondrocytes and chondroblasts, d) the papillary aspect e) the haemorrhagic areas, f) the hyalinization areas, g) fibroblast proliferation, H-Ex staining, x 140.

In II group the intima is moderately pronounced (fig. 6), a) with different thickness, it is poorly shaped, b) the muscle fibers are in the fibrous tunic. Hematoxelin-eosin staining shows (Fig. 7) early degeneration changes of intervertebral disc with the myxoid degeneration (a) the chondrocytes and chondroblasts could be seen in the center (b).

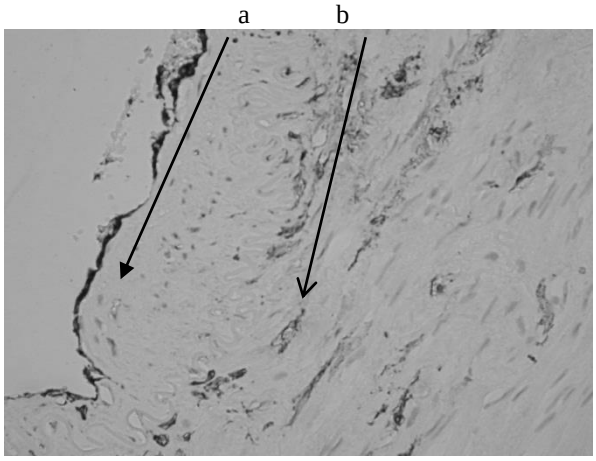

Figure 6. A 42-year-old patient B. The 5 lumbar vertebral artery a) the intima is sometimes interrupted, thinned with the irregular outline of the fibrillar structures b), CD34 IHC staining, x140.

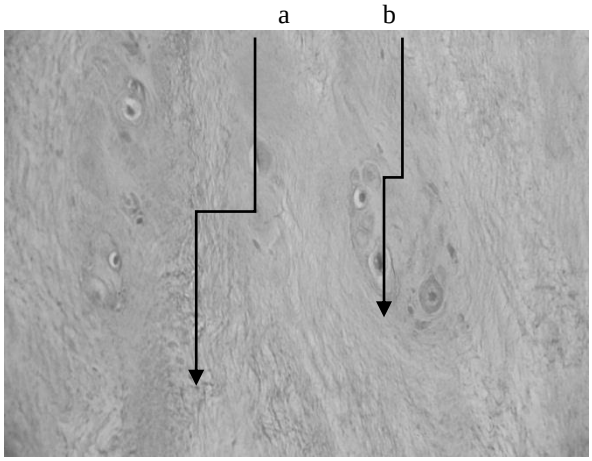

Figure7. a) the myxoid degeneration, b) the chondrocytes and chondroblasts are in the center, H-E staining, x 140.

The first group with positive intima (Fig. 8) the immunohistochemical staining shows a) the vascular intima is well defined, b) the persistence of structural elements in average tunic and adventitia; haematoxylin-eosin staining shows (Figure 9) : a) the chondrocytes and chondroblasts (b) the unchanged with well defined structural fibrillar structures (c), the persistence of fundamental condrogen substance (d).

IV. RESULTS AND DISCUSSION

Currently,the general objectives of the treatment of degenerative-dystrophic changes would be both to alleviate painful symptoms and to restore the mechanical functions. Depending on the degree of degeneration as a treatment strategies are designed to act as regenerative or restorative effect. The cell, gene and protein therapy have a regenerator effect, are most effective in early stage of the degenerative process, located in pulposus nucleus [17, 18, 26, 27, 28].

The cell therapy with mesenchymal stem cells plays a significant role in regeneration, being introduced locally in the degenerated disc, aimed, these cells proliferate in specific cells of disc with the same phenotype and as a result improve the degenerative effects, and lead to the production of extracellular matrix designed to restore the function of healthy disc [17, 26]. The repairing treatment is appropriate for more advanced stages of degeneration, which are characterized by the structural degradation of pulpous nucleus and annulus fibrous [16, 22, 23, 24]

The injection of the growth factors proteins as (BMP-7), β (TGFβ) leads to the transformation of tissue having an metaplasial effect, the cell differentiation with 5 (GDF-5) and others, all of which are widely studied, stimulating an extracellular production of matrix and a cell proliferation [18]. The injected directly into the disc of the anabolic factors effect are limited because of the short half-life and its rapid diffusion.

In animal models, the degeneration of intervertebral disc was reduced or even treated [18]. However, the given method is difficult to use clinically because it is a long process with progressive character [14]. Alternatively, the cell regeneration of disc can be manipulated by gene therapy, which involves the introduction of genes by viral-mediated vectors [27].

The tissue engineering has a significant effect in the regeneration of disc, although there are several non-biological methods, the cells generated by tissues preserve its remodeling and growth ability. The dominant paradigm of tissue engineering is that cells on a biomaterial or scaffold substrate can induce formation of new tissue stimulated (biological or physical) respectively. The hydrogels, such as alginate, hialuronan - collagen gels based, it was proved, support the survival of mature nucleus pulpous and promote extracellular matrix formation [23, 24]. Although, the engineering of pulpous nucleus and fibers of the annulus tissues was a special focus throughout the years, by increasing of discs (in vitro), including the translation of these technologies in large animal models (bovines), for the clinical trials, respecting the nutritional requirements (in vivo)[15, 19, 22].

The identification of growth factor TGFβ3 has a precursor effect on the cells of annulus fibers, during morphogenesis, he actively participates in the restoration of architecture and function after the degeneration of annulus fibers. In the intervertebral discs in rats, was found that TGFβ3 maintains the cell viability and improves the extracellular matrix in vitro [25]. Furthermore, the application of TGFβ3 on the adult cells of bovine fiber annulus cultured in the polymer nanofibers led's to the production of extracellular matrix with the optimizing of mechanical properties in vitro [20]. These nanofibers have a laminar structure, which unlike the native, the collagen fibers are wrapped in macromolecular chains located coiled with the dampers effect and in the intervertebral segment-flexion and torsion [21, 24].

V. CONCLUSION

Throughout many years of research the etiology of lumbar discogenic pain remains poorly understood, and the palliative therapies do not restore the structure of healthy disc or the mechanical function. The developing of intervertebral disc requires a merging of different cell types with some nutrients and oxygen, under the leadership of some complex molecular interactions. The cellular structures of pulposus nucleus and fiber annulus must work in an environment synergistically. The continue mechanical disturbances and poor nutrient supply of this throughout life induce the degenerative changes of disc. It is possible that early postnatal changes, including the vascular regression, modifies the cell phenotype of nucleus pulpous and the altered composition of extracellular matrix with biochemical and biomechanical changes of disc slowly, and irreversible. To establish the postnatal mechanisms of disc degeneration need a special attention in the future. The establishing of extracellular matrix synthesis has a significant role on this process. The investigations performed on vessels show its atherosclerosis.

With CD34 immunohistochemical study shows that vascular intima is better preserved in lumbar vertebral arteries in this case it is weak positive (5%); in four cases, moderately pronounced (20%); in 15 cases-is pronounced (75%), and the most affected is the five vertebral artery, intimate lacking vascular event (5%), 3 cases, poorly marked, in 10 cases, moderately pronounced (50%), and 6 cases-preserved. The atherosclerosis with vascular wall thickening affects the viability of structural elements in the intervertebral disc significantly. Initially, the nucleus pulpous is affected, then through a series of splitting mechanisms the collagen fibers become fragmentated that induce fibroblast proliferation with sclerosis.

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