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Research Paper

Qualitative and quantitative assessment of collagen and elastin in annulus fibrosus of the physiologic and scoliotic intervertebral discs

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ABSTRACT

The biophysical properties of the annulus fibrosus of the intervertebral disc are determined by collagen and elastin fibres. The progression of scoliosis is accompanied by a number of pathological changes concerning these structural proteins. This is a major cause of dysfunction of the intervertebral disc.

The object of the study were annulus fibrosus samples excised from intervertebral discs of healthy subjects and patients treated surgically for scoliosis in the thoracolumbar or lumbar spine. The research material was subjected to structural analysis by light microscopy and quantitative analysis of the content of collagen types I, II, III and IV as well as elastin by immunoenzymatic test (ELISA). A statistical analysis was conducted to assess the impact of the sampling site (Mann–Whitney test, $\alpha=0.05$) and scoliosis (Wilcoxon matched pairs test, $\alpha=0.05$) on the obtained results.

The microscopic studies conducted on scoliotic annulus fibrosus showed a significant architectural distortion of collagen and elastin fibres. Quantitative biochemical assays demonstrated region-dependent distribution of only collagen types I and II in the case of healthy intervertebral discs whereas in the case of scoliotic discs region-dependent distribution concerned all examined proteins of the extracellular matrix. Comparison of scoliotic and healthy annulus fibrosus revealed a significant decrease in the content of collagen type I and elastin as well as a slight increase in the proportion of collagen types III and IV. The content of collagen type II did not differ significantly between both groups. The observed anomalies are a manifestation of degenerative changes affecting annulus fibrosus of the intervertebral disc in patients suffering from scoliosis.

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1. Introduction

Scoliosis is one of the most common disorders of the spine. The most popular form of scoliosis is adolescent idiopathic scoliosis (AIS). AIS is a type of scoliosis that begins in people over 10 years of age. The overall prevalence of AIS is 0.47÷5.2%. The female to male ratio ranges from 1.5:1 to 3:1 and increases substantially with age (Asher and Burton, 2006; Konieczny et al., 2013). There are different hypotheses about the causes of this disease, including: muscular theory, neurological theory, connective tissue theory, bone growth mismatch theory, and genetic theory (Hefti, 2013; Yagi et al., 2014); however, the exact pathogenesis of AIS remains unknown. This disease most often affects the thoracic spine and is less common in thoracolumbar and lumbar spine.

The primary symptom of scoliosis is unilateral bulging of intervertebral discs (IVD) associated with the geometric deformity of the spine. It leads to remodelling in the outer part of the IVD within the annulus fibrosus (AF). The annulus is made mostly of mechanically strong fibrous proteins and acts as a protective covering of the soft inner core – nucleus pulposus (NP). Within normal and mature AF there can be 9 types of collagen (types: I, II, III, V, VI, IX, XI, XII, and XIV). Collagens types I and II are the most abundant (Yu et al., 2002), and they form closely adjoining lamellae in the amount of a minimum of 15 (posterior part of the AF) to a maximum of 25 (lateral part of the AF), concentrically arranged around the NP. The collagen fibres within each lamella are arranged parallel to each other and in each consecutive lamella are oriented obliquely with alternating directions (in opposite directions in adjacent lamellae) (Cassidy et al., 1989; Marchand and Ahmed, 1990; Pezowicz et al., 2005; Pezowicz et al., 2006). Such alternating arrangement of fibres in individual lamellae, called the angle-ply architecture (Cassidy et al., 1989), determines the very high strength of the IVD structure (Żak and Pezowicz, 2013) during the carrying and transmitting of dynamic compressive load throughout the spine column as well as providing spinal flexibility during bending and twisting (White and Panjabi, 1990).

The AF contains another structural protein, which, like collagen, has the ability to transfer mechanical loads. This protein is elastin (Han et al., 2015; Smith et al., 2008; Smith and Fazzalari, 2009; Yu et al., 2007). In many works on the biomechanics of the IVD and the AF the importance of elastin is neglected (O'Connell et al., 2012) and the majority of histological, biochemical and biomechanical studies have focussed on the roles of other extracellular matrix constituents, such as collagens and proteoglycans. This deficit may be the consequence of the perceived sparseness of elastin fibre distribution and its low relative percentage of the total tissue weight (Cloyd and Elliot, 2007; Mikawa et al., 1986; Olczyk, 1994) both within and between lamellae (Melrose et al., 2008; Schollum et al., 2008). Furthermore, elastin may be masked by the dense proteoglycan and collagen network (Yu et al., 2002). Most hypotheses regarding the importance and role of elastin show that it may play a critical role in reinforcing the mechanical integrity of the extracellular matrix under bending and torsion of the AF and in the recovery of the disc size and shape after deformation through facilitating its elastic recoil (Smith et al., 2008; Yu et al., 2002). Moreover, disorganisation of elastic fibres of the AF could contribute to the progression of spinal deformity (Yu et al., 2005). It was indicated that fatigue-related damage of elastin fibre connections between annulus lamellae is often manifested as separation of those lamellae and, consequently, over time, may lead to NP prolapse (Smith and Fazzalari, 2009).

The composition and network architecture of the extracellular matrix (ECM) of the AF determine the unique mechanical properties of the IVD, which are essential for the correct mechanical functioning of the whole spinal column. Remodelling of the IVD related to ageing, injury, or disease is characterised by a number of morphological changes in the disc, which are linked to the degradation of the extracellular matrix. It is worth noting that the boundary between physiologic IVD ageing and degenerative disc disease is not always clear, mainly due to the fact that ageing and degenerative changes do not substantially differ (Galbusera et al., 2014). Disc degeneration has been defined as an accelerated ageing process including structural failure (Adams and Roughley, 2006). In general, IVD degeneration is characterised by microstructural disarrangement of the AF extracellular matrix. The IVD undergoes irreversible chemical and structural changes, such as loss of collagen with an increase in the collagen type I to II ratio, decrease of proteoglycans and elastin, and increase of enzymatic activity (Han et al., 2012). Degenerative changes within the composition and network architecture of the extracellular matrix have a negative impact not only on the functional properties of the AF but also on ensuring transmission of the appropriate mechanical signals to the disc cells and IVD cell activity (Bruehlmann et al., 2004; Yu et al., 2007). Therefore, this study attempts to define the effects of degenerative changes caused by the development of scoliosis with respect to both the architecture and the quantitative composition of extracellular matrix proteins. There is still a lack of information about detailed quantitative characteristics of different types of collagen and elastin in the AF of the IVD, especially in the context of their mutual special relationships. The lack of data in this area makes it impossible to fully understand the role of collagen fibres and, especially, elastin fibres in the functioning of a healthy IVD as well as the development of degenerative disc changes in the course of scoliosis. Therefore, the aim of this study was a qualitative, quantitative, and morphological comparative analysis of fibrous structural proteins in the AF of normal IVDs and IVDs degenerated as a result of development of idiopathic scoliosis.

2. Materials and methods

2.1. Materials

The research material consisted of IVDs collected from young donors (less than 20 years old), both male and female. The AFs were obtained intraoperatively from 11 female patients treated surgically for thoracolumbar or lumbar scoliosis with the anterior approach. The age of patients ranged from 13 to 16 years old. The Cobb angle of the major scoliosis curve was 40–75 degrees (mean 53.4±10.4 degrees). Detailed patient