USE OF APPARENT DIFFUSION COEFFICIENT IN DISC DEGENERATION

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INTRODUCTION: Much work has been performed to characterize degenerative disc disease including gross morphologic, biochemical and histologic grading schemes (1,3,4). To better understand and treat disc degeneration, the responsibility has been placed on researchers to develop an accurate and non-invasive diagnostic tool able to objectively detect changes in the matrix with ageing and degeneration. Quantitative MR allows for an objective and reproducible way of assessing the MR sensitive protons and their interaction with matrix macromolecules. Our group has been able to correlate T1, T2, and magnetization transfer quantitative MR techniques to disc biochemical content and found that all these MR parameters were intimately linked to both the matrix biochemical makeup and grade of disc degeneration(2). Thus, we attempted to identify an MR technique that would be able to directly quantify matrix integrity, in this case percent collagen denaturation. The apparent diffusion coefficient or ADC is an MR technique which is capable of measuring diffusion directly from proton displacements. This theoretically allows for the study of matrix ultrastructure without the secondary effect of matrix subunit magnetic interaction. The aim of this study is to biochemically determine whether ADC is a reflection of matrix composition, integrity or both. To this end we performed in situ quantitative ADC assessments and compared these to matrix content and integrity (total collagen, GAG, water contents and % denatured total collagen).

METHODS: Fourteen human lumbar spines were obtained from fresh cadaveric specimens (within 18 hours of death). Extensive exclusion criteria were followed to obtain spines from normal and purely degenerative specimens. The anterior segment of the lumbar spine (L1 to S1) was retrieved. A total of 69 intervertebral discs were examined. Eight males and six females were used (mean age 52.9 ± 16). Our previous work has proven that there is no inter-disc variation in turnover phases, so each disc has been analyzed as a readily comparable specimen (1). The discs were cut into sagittal slabs and graded using the Thompson gross morphologic scheme (4). The specimens were then embedded in paraffin prior to performing MR analysis. Four standard solutions of 19.2 and 38.6 mM MnCl2, and 2% and 4% agar were attached to the samples. ADC was determined along the anterior/posterior axis using an SE sequence (TE 25ms, TR 700ms, TD 150 ms, Diffusion Gradient 0-24 mT/m). Specimens (2x3x5mm) were then isolated from the areas of interest of each disc (anterior annulus, nucleus pulposus, and posterior annulus). All specimens underwent chymotrypsin, and proteinase K treatments to quantitate total percent denatured collagen modifying a previously described technique (1). The extract material was also used to quantify GAG content (µg/mg) using the DMMB (dimethylmethylene blue) dye binding colorimetric assay. Adjacent specimens were dried at 110 °C for 4 days to obtain the dry weight. The dried samples were then hydrolyzed overnight with 6N HCl at 110°C prior to performing a colorimetric assay to evaluate the hydroxyproline content (µg/mg).

RESULTS: The age distribution ranges from 18-77 years (mean=48.2). ANOVA analyses revealed a significant rise in collagen denaturation with increased gross morphologic grade (p<.01) (graph 1). ANOVA demonstrated higher levels of denatured collagen in the nucleus than the annulus and these levels increased with increasing grade of degeneration (p<.01). These differences in collagen denaturation noted with increasing grade and degeneration depict the inhomogeneous nature of the disc and were demonstrated by our group previously (1). When relating ADC to water, GAG and collagen contents, we noted the pattern shown in graphs 2 and 3. The r values are measured at .211 and .1 respectively and demonstrate that ADC is not related to content of matrix subunits alone. In addition, relating ADC to %denatured collagen revealed the weak regression pattern shown in graph 4. When one performs a multiple logistic regression analysis an r of .694 is obtained (p=0.001) with the two most influencing variables being percent denatured collagen and proteoglycan.

DISCUSSION: ADC is not solely a reflection of the disc’s biochemical subunits or percent collagen denaturation. When a multiple logistic regression analysis is performed, taking into account the matrix composition and ultrastructural state (disc grade of degeneration and location), the apparent diffusion coefficient is a combined reflection of collagen denaturation and proteoglycan content. With our data, a model can be postulated to determine ADC:

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ADC (10^{-3} \text{mm}^2/\text{s}) = 1.05 + 4.42 \times 10^{-4} \times \text{GAG (µg/mgdw)} - 0.01 \times \% \text{coll. den.}
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This formula demonstrates that ADC is directly related to proteoglycan content and inversely related to collagen denaturation. This is the first correlation of its kind. It seems quite logical that proton diffusion would be affected by the varied ultrastructural state of the disc (reflected by collagen denaturation) and the quantity of proteoglycan molecules present (most prevalent matrix component). Such information can potentially be used in combination with quantitative T1, T2 and MT parameters to non-invasively obtain a quantitative assessment of disc matrix composition and structural integrity. This represents a significant advancement in our attempt to further MR’s role as a powerful diagnostic tool of disc degeneration.

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