

Characterization of Tissue Protein Profiles from Intervertebral Discs to Distinguish Symptomatic Patients from Asymptomatic Donors

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Introduction: Intervertebral disc (IVD) degeneration (IVDD) has been associated with pain and disability in patients. While IVDD is often associated with clinical signs and symptoms, numerous studies have indicated that IVDD occurs in many asymptomatic individuals at similar prevalence as with symptomatic patients. It is still unclear as to why some individuals develop symptomatic IVDD and others with similar severity of IVDD remain asymptomatic. This study was designed to assess and characterize the concentrations of inflammation- and degradation-related proteins from IVDs recovered from symptomatic (SYM) clinical patients and asymptomatic (ASYM) tissue donors to determine if differences in IVD protein profiles are associated with the development of symptomatic IVDD. It was hypothesized that the IVDs from SYM patients would have significantly higher levels of pro-inflammatory and pro-degradative biomarkers compared to IVDs from ASYM donors, and that the number of significant differences between SYM and ASYM cohorts would decrease with greater severity of IVDD.

Methods: Donor and Surgical Patient IVD Specimen Collection and Processing: With IRB approval (IRB#2010692) and informed patient consent, IVD tissues were recovered from SYM patients undergoing surgery for IVDD (n=20). With consent recorded in a legal permit under the Uniform Anatomical Gift Act, IVD tissues were recovered from qualified ASYM tissue donors (n=16) without a reported history of back pain. Level of IVD degeneration was determined using the Thompson grade gross assessment (ASYM) or Pfirrmann MRI grading system (SYM) using pre-surgical images. Tissue explants for each SYM patient, and annulus fibrosus (AF) and nucleus pulposus (NP) tissues explants from ASYM donors were weighed, frozen on the day of arrival in the laboratory at -80°C. For tissue protein extraction, IVD tissues were powdered in LN₂, and the protein content of the tissue was extracted using the T-PER Tissue Protein Extraction Reagent with protease inhibitors included. The protein content of the tissue extract was determined using BCA assay, and samples were tested for IL-6, IL-8, Gro- α , MCP-1, MCP-3, MIP-1 α , MIP-1 β , RANTES, TNF- α , PDGF-AA, VEGF, MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13 using Luminex assays. **Statistical analysis:** The concentration of each biomarker was standardized to the protein content of the extract for analysis. For ASYM AF and NP tissues were assessed separately, or as all tissues grouped together. Comparisons between SYM and ASYM tissues were determined when grouped based on symptomatic state only (SYM vs ASYM) or when separated by level of IVDD degeneration (grade 3 or ≥ 4). Significant differences ($p < 0.05$) between SYM and ASYM tissues were determined using a Mann-Whitney Rank Sum test or Kruskal-Wallis test with Bonferroni correction based on number of groups being compared.

Results: Differences in SYM and ASYM IVD Tissues Biomarker Concentrations (Fig. 1): SYM IVDs had significantly higher MCP-1, RANTES, PDGF-AA, VEGF, MMP-1, MMP-7, MMP-8, MMP-9, and MMP-13 compared to ASYM IVDs (ALL, AF, NP). SYM IVDs had significantly lower MCP-3 compared ASYM NP but not AF. **Differences in SYM and ASYM IVD Tissues Biomarker Concentration Grouped by Degradation Grade (Fig. 2):** For IVD tissue samples with degradation grade 3: SYM IVDs had significantly higher MCP-1, RANTES, PDGF-AA, MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13 compared to all ASYM tissues (ALL, AF, NP). SYM IVDs also had significantly lower MCP-3 compared to the ASYM-ALL and ASYM-NP groups. For the IVD tissue samples with degradation grade ≥ 4 , the SYM group had significantly higher MCP-1, PDGF-AA, VEGF, and MMP-9 compared to the ASYM-ALL and ASYM-AF groups, and MMP-7 compared to the ASYM-ALL and ASYM-NP groups.

Discussion: The data from this study indicate that IVDs from clinical patients with symptomatic IVDD are associated with significantly higher inflammatory and degradative protein concentrations compared to IVDs recovered from ASYM donors. Additionally, the protein profile differences between SYM and ASYM IVDs were more evident at earlier stages of degeneration based on the number of biomarkers with significantly higher concentrations in SYM tissues with grade 3 degradation compared to tissues with grade 4/5 degradation. Therefore, increased inflammatory signaling and degradative enzyme activity at the earlier stages of IVDD may be important distinguishing factors in the development of symptomatic IVDD. However, further study is required to determine if these changes in IVD protein profiles are directly linked with development of symptomatic IVDD or are a consequence of other variables. Ongoing studies in our lab are aimed at further characterization of SYM and ASYM IVDs to determine direct links to development of symptomatic IVDD that may serve as biomarkers for clinical application.

Significance: The characterization of IVD protein profiles performed in this study delineated significant differences between symptomatic patients and asymptomatic donors that may be related to development and severity of symptomatic IVDD. Understanding factors that are associated with the development and progression of symptomatic IVD degeneration may allow for the development of novel diagnostic, preventative, and treatment methodologies for patients.