Relationships among Patient Demographic Factors and Biomarkers in Infrapatellar Fat Pads Recovered from Osteoarthritic Knees

Kathleen A. Harris, Kayla R. Schweitzer, Cole C. Stevens, Ahmed S. Fouad, James A. Keeney, James L. Cook, Aaron M. Stoker
Thompson Laboratory for Regenerative Orthopedics, University of Missouri, Columbia, MO
stokera@health.missouri.edu

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Introduction

Osteoarthritis (OA) is a painful and irreversible joint disease that is a significant cause of disability in patients worldwide. OA is a disorder of the entire "joint organ" with all tissues contributing to, and affected by, the development and progression of disease. Previous studies have suggested that the knee's infrapatellar fat pad (IPFP) may significant roles in the development and progression of OA. Additionally, patient demographics such as age, sex, and BMI have been associated with increased risk for OA. However, the relationships among patient demographics and OA-related biomarkers in the IPFP from osteoarthritic knees have not been fully elucidated. This study was designed to identify potential relationships among patient age, sex, BMI and self-reported visual analog scale (VAS) pain levels and the concentrations of inflammatory and degradative proteins in the IPFP from patients undergoing total knee arthroplasty (TKA) for treatment of symptomatic knee OA. It was hypothesized that significantly higher IPFP pro-inflammatory and pro-degradative tissue protein concentrations would be observed in female patients of older age, higher BMI and more severe pain.

Methods

Tissue collection: With IRB approval (IRB# 1208392) and informed patient consent, resected IPFP tissues that would otherwise be discarded after surgery were recovered from the knees of 30 OA patients (mean age 64.6 years, sex: 24 female, 6 male, mean BMI 33.82) undergoing total knee arthroscopy (TKA). One tissue explant (6mm diam) of the IPFP was created using a dermal biopsy punch and stored at -80°C. *Tissue Protein extraction*: The protein content of the tissue samples was extracted using the T-PER protein extraction reagent (Fisher) with protease inhibitors included. The IPFP tissue explants were homogenized using a mini-bead beater, the homogenate was centrifuged to pellet tissue debris, and the supernatant was stored at -80°C until used for analysis. *Protein Biomarker Analysis:* The BCA assay was used to determine the protein concentration of the tissue extract and media. The concentration of MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, GRO-α, MCP-1, MCP-3, PDGF-AA, IL-6, IL-8, MIP-1α, MIP-1β, RANTES, TNF-α, VEGF, Leptin, Adiponectin, Adipsin, CRP, and Resistin were determined using commercially available Luminex assays according to the manufacturer's protocol. *Statistical analysis:* The concentration of each biomarker was standardized to the protein content of the tissue extract and log transformed for analysis. Patients were divided into groups based on BMI (≤25, 26-30, 31-35, 36-39, ≥40), age (<60y, 60-65y, 66-69y, ≥70), sex (M, F), and VAS pain at the time of surgery (0, 2, 3-4, 5-10). Significant ($p<0.05$) differences in IPFP tissue biomarker concentration based on patient BMI, age, and VAS pain groups were determined using one-way ANOVA and Tukey post-hoc test, and between male and female patient using a T-test. Differences in IPFP tissue biomarker concentration based on patient demographic groups, when accounting for the other demographic factors, were determined using a univariate linear mixed model.

Results

Effects of Patient Age (Fig. 1): The concentration of MMP-3 was significantly higher in patients aged 66-69y compared to younger patients, and the concentration of Adiponectin was significantly higher in patients younger than 60y compared to patients older than 65y. When accounting for the other patient demographic factors, the concentration of MMP-3 was significantly higher in patients older than 65 compared to patients younger than 60y. Accounting for patient demographic factors did not change the relationship between patient age and the concentration of Adiponectin in the OA IPFP. ***Effects of Patient Sex (Fig. 2):*** The concentrations of MMP-2, MMP-9, GRO-α, MCP-3, MIP-1α, MIP-1β, and VEGF were significantly higher, and the concentration of IL-6 was significantly lower, in female patients compared to male patients. When accounting for the other patient demographic factors, there was not a significant difference in the concentration of these proteins based on the sex of the patient. However, OA IPFP recovered from female patients had significantly higher concentrations of Resistin compared to male patients when accounting for the other patient demographic factors. ***Effects of Patient BMI:*** No significant differences in the IPFP protein concentrations were identified when grouped based on patient BMI. ***Effects of Patient VAS Pain:*** The concentration of MMP-3 was significantly higher in patients with a VAS pain score of 2 compared to patients with a VAS pain score of 3-4 and 5-10 at the time of surgery when accounting for the other patient demographic factors.

Discussion

The data from this study elucidated significant differences in targeted biomarker concentrations in IPFP of individuals with knee OA based on patient age, sex, and self-reported pain levels. Patient age was associated with significant differences in MMP-3 and Adiponectin. Self-reported pain severity was also associated with differences in MMP-3 in the IPFP, with older individuals and patients with lower severity pain having higher IPFP concentrations of MMP-3. Since MMP-3 can activate other MMPs, this pathway may be particularly important as a target for intervention. IPFPs from female patients had higher concentrations of specific degradative enzymes and chemokines compared to males. However, when the other patient demographic factors were considered (age, BMI, pain), the significant differences between males and females for these proteins were not observed. Interestingly, patient BMI did not account for significant differences in targeted biomarkers from IPFP as analyzed in this study.

Significance

The results of this study highlight the importance of considering patient variables when assessing metabolic changes in tissues that contribute to the development and progression of OA. Unraveling the complex relationships among patient factors and pathomechanisms will allow for the development of novel patient-specific assessment and management strategies that improve outcomes for people suffering with OA. Ongoing studies in our lab are aimed at further elucidation of these relationships toward elucidation of whole-organ pathomechanisms that can be targeted for advancing more effective preventative and therapeutic strategies for OA.