Relationships among Patient Demographic Factors and Biomarkers in Synovium Recovered from Osteoarthritic Knees

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Introduction

Osteoarthritis (OA) is a significant cause of disability in patients, characterized by pain, effusion and dysfunction of the affected joints. The joint is considered an organ, such that the synovium tissue (SYN) in the knee plays a significant role in the development and progression of knee OA by releasing pro-inflammatory and pro-degradative biomarkers. While the concentration of these biomarkers varies between patients, it is unclear if patient demographic factors influence SYN tissue biomarker concentrations. Therefore, this study was designed to determine if patient demographic factors, including age, sex, BMI, and self-reported visual analog scale (VAS) pain levels, are related to differences in SYN tissue inflammation and degradative enzyme related biomarker concentrations from patients undergoing total knee arthroplasty (TKA) for symptomatic knee OA. It was hypothesized that as pain, age, and BMI increase, the levels of pro-degradative and pro-inflammatory biomarkers in the SYN of TKA patients would significantly increase. Further, it was hypothesized that the concentration of pro-degradative and pro-inflammatory biomarkers in the SYN of female TKA patients will be significantly greater than in male TKA patients.

Methods

Tissue collection: With IRB approval (IRB# 1208392) and informed patient consent, SYN tissues normally discarded after surgery were recovered from the knees of 31 OA patients (mean age 63.46 years, sex: 25 female, 6 male, mean BMI 34.12) undergoing TKA. One tissue explant (6mm) of the SYN 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 SYN 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 for analysis. Patients were divided into groups based on BMI (≤25, 26-30, 31-35, ≥36), age (<60y, 60-65y, 66-69y, ≥70), sex (M, F), and VAS pain at the time of surgery (0, 1-2, 3-4, 5-10). Significant ($p<0.05$) differences in SYN 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 SYN tissue biomarker concentration based on patient demographic groups when accounting for the other demographic factors were determined using a univariate linear mixed model.

Results

Effect of Patient Age: No significant differences in OA SYN tissue protein content were identified based on patient age. ***Effect of Patient Sex: (Fig. 1)*** The concentration of TIMP-1, VEGF, Adiponectin, and Adipsin in the SYN were significantly higher in female patients compared to male patients. When accounting for the other patient demographic factors, the concentration of TIMP-1, Adiponectin, and Adipsin were still significantly higher in females than males, and patient age was significantly associated with the difference in the concentration of Adipsin in the SYN based on sex. ***Effect of Patient BMI (Fig. 2):*** Patients with higher BMI had significantly higher concentrations of TIMP-2 and Leptin, and significantly lower concentrations of MIP-1α, in the SYN compared to patients with lower BMI. When accounting for the other patient demographic factors, the significant differences in the concentration of TIMP-2, Leptin, and MIP-1α in the SYN based on BMI groups were still observed, but the other demographic factors were not significantly associated with the difference in SYN protein concentration based on patient BMI. ***Effect of Patient VAS Pain:*** No significant differences in OA SYN tissue protein content were identified based on the VAS pain of the patient alone. However, when accounting for the other patient demographic factors, the concentration of MIP-1α, VEGF, TIMP-1, and TIMP-3 in the SYN of patients with a VAS pain score of 1-2, and Adiponectin for patients with a score of 1-4, was significantly higher than for patients with a VAS pain score of 0 and 6-10 this study. Patient sex was significantly associated with the concentration of MIP-1α, VEGF, TIMP-1, and Adiponectin, and BMI for the concentration of Adiponectin, in the SYN of OA patients based on VAS pain groups.

Discussion

The data from this study elucidated significant differences in targeted biomarker concentration in the OA SYN based on patient sex and BMI, and these demographic factors have a significant effect on the differences in OA SYN protein content based on VAS pain. This observation highlights the significant affect that patient demographic factors can have on the changes in inflammatory and degradative responses of joint tissues related to OA. The data indicates that patient sex and BMI are associated with differences in TIMP concentration and adipokine signaling in the SYN of OA patients. Since adipokines are considered part of a pro-inflammatory signaling cascade, the data from this study provides further evidence for adipokine signaling as a factor in the development and progression of OA, with patient sex and BMI significantly contributing to the levels of these signals in the synovium. On going studies are aimed at expanding on the data from this study, and determining how patient factors affect the SYN during OA development and progression. Determining the intersection between the effects of patient demographics and OA development on changes to the tissues of the OA joint may allow for the development of more patient specific treatment options to improve the outcomes for patients with OA.

Significance

The data from this study indicates the importance of sex and BMI when assessing the concentration of TIMPs and adipokines in the OA synovium. Unraveling the complex relationship between patient demographic factors and the development and progression of OA may allow for the development of novel patient specific treatment and assessment strategies that could result in improved outcomes for patients with OA.