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Changes of synovial fluid protein concentrations in supra-patellar bursitis patients after the injection of different molecular weights of hyaluronic acid

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ABSTRACT

Knee pain is commonly seen in orthopedic and rehabilitation outpatient clinical settings, and in the aging population. Bursitis of the knee joint, especially when the volume of the synovial fluid is large enough, can compress and distend the nearby soft tissues, causing pain in the knee joint. Out of all the bursae surrounding the knee joint, supra-patellar bursitis is most often associated with knee pain. Treatment strategies in managing supra-patellar bursitis include the aspiration of joint synovial fluid and then followed by steroid injection into the bursa. When supra-patellar bursitis is caused by degenerative disorders, the concept of viscosupplementation treatment may be effective by injecting hyaluronic acid into the bursa. However, the rheology or the changes in the concentrations of proteins (biomarkers) that are related to the development of bursitis in the synovial fluid is virtually unexplored. Therefore, this study aimed to identify the concentration changes in the synovial fluid total protein amount and individual proteins associated with supra-patellar bursitis using the Bradford protein assay and western immunoglobulin methods. A total of 20 patients were divided into two groups with 10 patients in each group. One group received the high molecular weight hyaluronic acid product of Synvisc Hylan G-F 20 and the other group received the low molecular weight hyaluronic acid product of Hya-Joint Synovial Fluid Supplement once per week injection into the bursa for a total of 3 weeks. Significant decreases in the synovial fluid total protein concentrations were observed after the second dosage of high molecular weight hyaluronic acid injections. Apolipoprotein A-I, interleukin 1 beta, alpha 1 antitrypsin, and matrix metalloproteinase 1 proteins revealed a trend of decreasing western immunoblotting band densities after hyaluronic acid injections. The decreases in apolipoprotein A-I and interleukin 1 beta protein band densities were significant in the high molecular weight hyaluronic acid injection group. Transthyretin, complement 5, and matrilin 3 proteins revealed a trend of increasing western immunoblotting band densities after hyaluronic acid injections. Transthyretin revealed significant increases in protein band densities in both the high and low molecular weight hyaluronic acid injection groups. This study may provide the rationale for targeting several biomarkers associated with lipid transport, inflammation, and anti-aging as possible disease modifying therapies for the treatment of supra-patellar bursitis and even degenerative joint disorders.

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1. Introduction

Knee pain is a common complaint in orthopedic and rehabilitation outpatient clinical settings. Bursitis of the knee joint, especially when the volume of the synovial fluid is large enough, can compress and distend the nearby soft tissues, causing pain in the knee joint (de Miguel Mendieta et al., 2006). Common clinical symptoms include local joint pain, stiffness, as well as possible burning pain. Patients suffering from supra-patellar bursitis may have trouble standing, and in performing activities such as standing, walking, squatting and running (Hill et al., 2001). Joint stiffness can also occur the next day after waking up from sleep (Hill et al., 2001).

There are several bursae surrounding the knee joint. These bursae include the supra-patellar bursa, also known as the supra-patellar pouch, infra-patellar bursa, bursa at the pes anserinus, and at the popliteal fossa area (e.g. Baker's cyst) (de Miguel Mendieta et al., 2006). The

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supra-patellar bursa is located between the quadriceps tendon and the femur and communicates with the synovial cavity of the knee (Marra et al., 2008). Bursae are synovium-lined structures usually not easily detected by any imaging method. Bursitis can be detected using arthrography, soft tissue musculoskeletal ultrasound, and can also be seen on routine magnetic resonance imaging (MRI) scans (Hayashi et al., 2010). Inflammation of the bursa or bursitis will result in a cystic-like appearance due to accumulation of the fluid within the bursa and thickening of the synovial membrane (Beaman and Peterson, 2007).

Out of all the bursae surrounding the knee joint, supra-patellar bursitis is most often associated with knee pain. Supra-patellar bursitis is significantly correlated with knee pain when soft tissue ultrasound measures a 2 millimeter (mm) distention of the bursa due to the presence of increased synovial fluid (SF) volume (de Miguel Mendieta et al., 2006). The cause of bursitis can be associated with inflammatory or degenerative arthritis (such as knee OA), infection, and malignancy (Hayashi et al., 2010). Osteoarthritis (OA) remains to be the frequent cause of supra-patellar bursitis as synovitis is a common manifestation observed in knee OA (Hedbom and Hauselmann, 2002). It is crucial to differentiate the causes of supra-patellar bursitis as the treatment strategy is different. For bursa that is infected, further investigation is needed as well as the initiation of antibiotic therapy (Aaron et al., 2011).

The most often performed treatment strategy in managing supra-patellar bursitis is the aspiration of joint SF followed by steroid injection into the bursa. Although reports have shown that the injection of steroid can increase the walking distance in patients with bursitis as compared with patients who did not receive the steroid treatment, the mechanism behind the treatment effectiveness of steroid remains controversial (Leung et al., 2011). The concept of viscosupplementation has been widely practiced clinically in the treatment of knee OA (Cohen et al., 2008). Viscosupplementation is a therapeutic modality based on the replacement of SF with a hyaluronic acid (HA) solution (Balazs, 2004). Based on our clinical experience, injecting HA solution into the supra-patellar bursa after aspiration can offer longer lasting analgesic effects and effectively reduce the volume of SF as compared with other treatment options such as oral non-steroid anti-inflammatory drugs, application of physical modality onto the affected knee joint, and steroid injection in a higher number of patients (Leung et al., 2011).

Most of the up-to-date studies related to intra-articular (IA) HA injections focus mainly on the regulation and expression of interleukin (IL) and tumor necrosis factor-alpha in fibroblast-like synoviocytes (Huang et al., 2011). The regulations or the changes in the concentrations of proteins (biomarkers) that are related to the development of bursitis remain virtually unexplored. In this study, laboratory techniques of protein assay and western immunoblotting will be used. Proteins that revealed significant concentration differences before and after HA injections on 2-dimensional electrophoresis (2-DE) gel analyses will be further validated using western immunoblotting. After the completion of this study, we hypothesize that there will be significant changes in the protein concentrations that are related to inflammation and oxidation. We hope that the results obtained in this study may help in the future development of novel treatment options for patients with supra-patellar bursitis, such as protein supplementation or developing chelating agents against certain biomarkers.

2. Materials and methods

2.1. Subjects

In this study, a total of 20 patients diagnosed with supra-patellar bursitis on the unilateral knee were recruited. The inclusion criteria were:

1. The thickness of the supra-patellar bursa was greater than 2 mm as confirmed by musculoskeletal ultrasound. Ultrasound has also confirmed that the supra-patellar bursa is in communication with the synovial cavity of the knee joint.
2. The volume of the synovial fluid (SF) in the supra-patellar bursa was the cause of pain in the knee joint.
3. The injection of hyaluronic acid (HA) into the bursa resulted in the reduction of SF volume and in the alleviation of pain for the patient.
4. Patient has received steroid injections, oral nonsteroidal anti-inflammatory drugs (NSAIDs), and physical modality treatments (e.g. shortwave diathermy and interferential wave) but without reduction in SF volume and the alleviation of joint pain.
5. The major cause of suprapatellar bursitis is due to degenerative knee disorder (e.g. knee OA) or causes other than infectious and inflammatory knee disorders.

Exclusion criteria included patients with history of a metal knee implant, pregnancy, severe degeneration of knee joints with total obliteration of joint space, joint and chicken or egg allergy (Tang et al., 2005). Patients with isolated supra-patellar bursa mass mistakenly diagnosed as bursitis was not enrolled in this study. The aspirated SF prior to HA injections was sent for SF analysis. SF showing evidences of crystals suggesting possible gouty arthritis and infection was excluded from the study. All patients signed the informed consent before participating in this study. The institutional ethics committee approved all the protocols involved in this study.

2.2. Sample collection

The aspiration technique followed the standard lateral approach with the knees extended. A strict sterilized procedure was applied to prevent septic infection. The musculoskeletal ultrasound was used to accurately guide the needle into the bursa for the aspiration of SF. Accurate placement of the needle in the bursa avoided poking the needle into the muscle or other soft tissues which may cause SF to be contaminated with blood (Chen et al., 2011a).

Each patient has different volumes of bursa fluid. As much SF as possible was aspirated from the bursa. Approximately 5 to 10 mL of the aspirated SF was sent for biochemical analysis first. Samples showing evidence of active infection or inflammation were not included in this study. SF samples were centrifuged at 2500 rpm for 20 min at 4 °C. The supernatants were stored in 90 μl aliquots with 10 μl of a proteinase inhibitor solution containing 100 mM EDTA (Sigma, St. Louis, MO, USA), 20 mM *N*-ethylmaleimide (Sigma), and 20 mM aminoethylbenzenesulfonyl fluoride (Sigma) added. SF samples free of infection, inflammation, and red blood cells were further aliquoted and stored at −20 °C if they were to be used for experimental analysis within one week. Otherwise the samples were stored at −80 °C. Total protein concentration was calculated for every SF sample.

2.3. Treatment protocols

The recruited 20 patients were divided into two groups with 10 patients in each group. One group received the high molecular weight hyaluronic acid (HMW HA) product of 2 mL Synvisc Hylan G-F 20 (Genzyme Biosurgery, USA) with 6000 kDa). The other group received the low molecular weight hyaluronic acid (LMW HA) product of 2 mL Hya-Joint Synovial Fluid Supplement (SciVision Biotech Inc., Taiwan) with 500–730 kDa. Patients received once per week injection treatment into the bursa for a total of 3 weeks. Ultrasound-guided approach was applied for accurate aspiration of bursa SF samples and the injection of HA into the supra-patellar bursa.

2.4. The calculation of SF total protein concentrations

The standard curve of known bovine serum albumin (BSA) (Sigma, >96% purity) concentrations (1 μg, 0.5 μg, 0.25 μg, and 0.125 μg) was constructed to calculate the total protein concentrations of the SF. The

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