Intercellular adhesion molecule (ICAM-1, CD54) is increased in capsule tissue and joint fluid of the patients with frozen shoulder

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Introduction

Adhesive capsulitis (frozen shoulder) is a very common cause of shoulder disability and pain. This disorder is characterized by dense fibrosis of the glenohumeral capsule, leading to a contracture of the rotator interval and the coracohumeral ligament, which restricts the shoulder motion. But the pathophysiology of this disorder is poorly understood and there are very limited reports regarding the cytokines and their expression in the frozen shoulder. In this study, we compared the expression of intercellular adhesion molecules (ICAM-1) of capsular tissue derived from patients with frozen shoulder to normal controls.

Materials and Methods

Glenohumeral capsule tissue was obtained intraoperatively from patients with idiopathic adhesive capsulitis during surgery. Human tissues were obtained with the approval of the Catholic University School of Medicine Institutional Review Board.

RNA Extraction and Oligo-Array Analysis for Gene Expression

20 patients were candidates for this study. They were divided into two groups: 15 patients with adhesive capsulitis and 5 normal controls from the patients with proximal humerus fracture. Capsule tissues were obtained during the surgery and stored in the RNAlater® (Ambion, CA) at −20°C. Total RNA from the capsule tissues were extracted using TRIZOL (Invitrogen, Rockville, MD) according to the manufacturer's instructions. GEArray® Series (Bioscience Corporation, Fredrick, MD) for Human Extracellular Matrix & Adhsion Molecules were used.

Gene detection in capsulel tissue by real time reverse transcription-polymerase chain reaction (RT-PCR)

We can confirm the difference of gene expression between the capsule tissue from frozen shoulder (15 patients) and the normal capsule tissue (5 patients) from the fracture and instability patients. Real time PCR was performed up to 40 cycles using the SMART Cycler (Cepheid, Sunnyvale, CA) and Syber Green dye. Each sample were tested in duplicate and 18s gene was used as reference gene.

ICAM-1 detection in the joint fluid of the frozen shoulder patient by western blotting

Joint fluid was extracted from the patients with frozen shoulder (7 patients) and anterior instability (2 patients). Anti-human ICAM monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) was used. Staining was detected with an chemiluminescence kit (Amersham Biotech, Arlington Heights,IL), and quantified by densitometry with Image Analyzer LAS-3000 Multi Gauge software (Fujifilm corporation , Tokyo.Japan).

Statistical analysis

Student t-test and Mann-Whitney U test were used for comparison between the frozen shoulder and control groups. P-values less than 0.05 were considered significant.

Results

RNA Extraction and Oligo-Array Analysis for Gene Expression

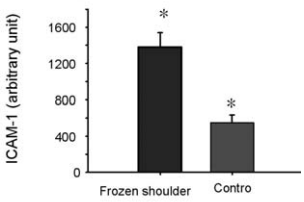

Fig. 1. The gene expression of ICAM-1 was significantly increased in Group I (frozen shoulder) compared to Group II (control capsule) ($p<0.05$). The average relative intensity of expression of frozen shoulder group was 14666.44 unit and those of control was 5881.22 unit. ICAM-1 gene is expressed as a bright square dot on the membrane of capsule from the patient with frozen shoulder, while there is no expression of ICAM-1 gene on the membrane of control sample.

Gene detection in capsule tissue by real time reverse transcription-polymerase chain reaction (RT-PCR)

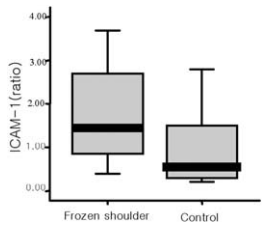

Fig. 2. Relative ICAM-1 expression in patients and control were estimated by quantitative real-time PCR. Expression levels of ICAM-1 mRNA were significantly higher in the patient with frozen shoulder compared to control ($p<0.05$). Average expression level was 1.698°æ0.186 in frozen shoulder group and 0.999°æ0.236 in control.

ICAM-1 detection in the joint fluid of the frozen shoulder patient by western blotting

The western blotting results revealed clearly that the expression of ICAM-1 protein in the glenohumeral joint fluid of the patients with frozen shoulder (7 patients) was increased definitely compared to control (2 patients). Molecular weight of ICAM-1 was 85~115 kDa.

Discussion & Conclusion

This study demonstrated that the gene expression of ICAM-1 was increased significantly in the capsule tissue of patients with frozen shoulder and this molecule was also increased in the joint

fluid of the patients with frozen shoulder compared to control tissue. The results imply this specific molecule might play an important role in fibrotic processes and causing the stiffness in adhesive capsulitis. Therefore, regulation of ICAM-1 expression might be a key of anti-adhesion agent for treatment of frozen shoulder. Future study should be focused on this point.