Elevation of MMP1 and ADAMTS5 mRNA expression in glenohumeral synovia of patients with hypercholesterolemia

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Abstract

Background: Epidemiological studies have reported a positive association between hypercholesterolemia and shoulder disease. Previous studies have focused on the effect of hypercholesterolemia on tendinopathy. Moreover, hypercholesterolemia has also been linked to joint pathology in the knee and hand. However, the effect of hyperlipidemia on glenohumeral joint remain unclear. A hypercholesterolemic condition has been reported to alter levels of A Disintegrin and Metalloprotease with Thrombospondin Motifs (ADAMTSs) and matrix metalloproteases (MMPs) in synovium of the knee joint. Here, we evaluated the mRNA expression of ADAMTSs and MMPs in the glenohumeral synovium of patients with and without hypercholesterolemia.

Methods: Study participants were 73 patients who underwent arthroscopic rotator cuff repair for degenerative rotator cuff tears. They were divided into two groups according to total cholesterol (TC) and triglyceride levels. Synovial membrane samples were harvested at the rotator interval during surgery, and mRNA expression levels of the aggrecanases ADAM-TS4 and ADAM-TS5 and MMPs (MMP-1, 3, 9, and 13) were analyzed quantitatively.

Results: ADAM-TS5 and MMP1 mRNA levels were significantly higher in the high TC group than in the low TC group (P = 0.023 and P = 0.025, respectively). In contrast, no significant differences were observed in ADAMTS4 or MMPs 3, 9, and 13 (ADAMTS4, P = 0.547; MMP3, P = 0.55; MMP9, P = 0.521; and MMP13, P = 0.785).

Conclusion: Hypercholesterolemia may alter MMP1 and ADAMTS5 expression in the synovium of the glenohumeral joint.

Keywords: Osteoarthritis, Hypercholesterolemia, ADAMTS5, MMP1, Synovium

Background

Several systemic factors have been linked with degenerative shoulder diseases, including dyslipidemia, diabetes and obesity [1–3]. Hypercholesterolemia is a systemic metabolic disease characterized by abnormally high levels of cholesterol in the blood which has effects on not only internal organs and cardiovascular tissues but also the musculoskeletal system [4]. Previous studies have focused on the effect of hypercholesterolemia on the rotator cuff [5–7]. However, an evaluation of comorbidities in patients presenting with shoulder osteoarthritis (OA) revealed that 48.7% of patients with primary shoulder OA also suffered from hyperlipidemia [8]. Hypercholesterolemia has also been statistically associated with glenohumeral joint pain but not rotator cuff tendinopathy [9]. Notably, hypercholesterolemia has also been linked with OA in the knee and hand [10, 11]. These
observations suggest that hypercholesterolemia affects not only tendons but also joint pathology in the shoulder. Nevertheless, the effect of hyperlipidemia on the glenohumeral joint remains unclear.

The matrix metalloproteinases (MMPs) and A Disintegrin and Metalloproteinase Domain with Thrombospondin motifs (ADAMTS) family play a critical role in the destruction of extracellular matrix in arthritis, rotator cuff disease and other musculoskeletal diseases [12–15]. Synovial tissue is a major source of MMPs and ADAMTS, the expression of which could be affected by dyslipidemia [16, 17]. We previously found that dyslipidemia altered synovial MMP and ADAMTS expression levels in a mouse model of knee OA that exhibited dyslipidemia [16]. Previous studies reported the elevation of MMPs in tendons in patients with tendinopathy [14] and that this elevation was affected by hypercholesterolemia [4]. However, it remains unclear whether dyslipidemia alters MMPs and ADAMTS expression in the synovium of the glenohumeral joint.

Here, we evaluated the mRNA expression of MMP and ADAMTS in the glenohumeral synovium of patients with and without dyslipidemia.

**Methods**

**Patients**

This study was approved by the Ethics Committee of our institution (Clinical Research Review Board of the Kitasato Institute; reference number KMEO B13-113) and abode by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants.

Synovial membrane samples acquired from the glenohumeral joint, specifically areas of the rotator interval showing redness, were obtained from patients who underwent arthroscopic surgery from November 2017 to October 2020. Patients with RCT were clinically assessed prior to surgery using the Constant score [18]. We graded radiographic OA as normal (no osteophytes), mild (< 3 mm), moderate (3–7 mm), or severe (≥ 7 mm) using the Samilson–Prieto classification [19]. All arthroscopic surgeries to repair a torn rotator cuff were performed by an experienced shoulder surgeon. We excluded patients with a history of rheumatoid arthritis, other collagen diseases, and fractures in the humerus or glenoid. The remaining patients were divided into two groups according to their total cholesterol (TC) level (< 220 mg/dl and ≥ 220 mg/dl), based on the standardized value established by the Japan Atherosclerosis Society (JAS) [20]. Patients were also divided into two groups according to triglyceride (TG) levels (< 150 mg/dl and ≥ 150 mg/dl), also based on the standardized value of the JAS [20].

**Statistical analysis**

Results are expressed as mean ± standard deviation (SD). Statistical significance was determined using the nonparametric Mann–Whitney U test or unpaired t-test. Statistical significance was set at P < 0.05. All statistical analyses were conducted using the SPSS software v19.0 (IBM, USA).

### Table 1 Sequences of primers

| Gene   | Direction | Primer sequence (5′–3′) | Product size (bp) |
|--------|-----------|-------------------------|------------------|
| ADAMTS4 | F         | AACACTGAGGGACTGCGGAC   | 159              |
|         | R         | AGGAGTTGTGACTGCGGCTT   |                  |
| ADAMTS5 | F         | ATGCACTTACGGCGCACATCA  | 114              |
|         | R         | TCGTAGGCTTGCCCGGGAG    |                  |
| MMP1   | F         | ACCTACATCGGTGGTGGCGC   | 164              |
|         | R         | CGAGGGCGTGGCAGGATT    |                  |
| MMP3   | F         | GTGGAGTCTCGAGTTGGTG    | 164              |
|         | R         | TGGAGTCACTCTCCCGGA     |                  |
| MMP9   | F         | TTGAGTCCGGTGAGGATG     | 197              |
|         | R         | GCTTCTCAAGAGCGAGGATCC  |                  |
| MMP13  | F         | TGACTGAGGGCGTTCCAGAGAA| 111              |
|         | R         | CATCAGGAACCCCGCATCTT  |                  |
Results

Expression of ADAMTS and MMPs in patients with low and high TC levels

The clinical characteristics of patients are summarized in Table 2. Briefly, 42 patients were assigned to the low TC group (27 men and 15 women, aged 65±9 years) and 31 patients were assigned to the high TC group (23 men and 8 women, aged 64±11 years; Table 2). In both groups, no significant differences were observed in patient age at surgery (P=0.505), men/women ratio (P=0.449), body mass index (P=0.539), TG levels (P=0.018), Constant score (P=0.668), or tear size (P=0.465). ADAMTS5 and MMP1 levels were significantly higher in the high-TC group than in the low-TC group (ADAMTS5, P=0.023; MMP1, P=0.025; Fig. 1b, c). In contrast, no significant differences were observed for ADAMTS4 and MMP3, 9, and 13 (ADAMTS4, P=0.547; MMP3, P=0.55; MMP9, P=0.521; MMP13, P=0.785; Fig. 1a, d, e, f).

Expression of ADAMTS and MMPs in patients with low and high TG levels

According to the criteria, 33 patients were assigned to the low-TG group (16 men and 17 women, aged 65±8 years) and 40 patients were assigned to the high-TG group (34 men and 6 women, aged 64±11 years; Table 3). No significant differences between groups were found in the average age of patients at surgery.

Table 2 Clinical characteristics of low and high total cholesterol (TC) groups

|                         | Low TC group | High TC group | P value |
|-------------------------|--------------|---------------|---------|
| Age during surgery (years) | 65.5±9.2     | 63.9±10.9     | 0.647   |
| Number of patients (men/women) | 27/15        | 23/8          | 0.449   |
| BMIa (kg/m²)             | 25.0±3.4     | 26.1±4.3      | 0.539   |
| Total cholesterol level (mg/dl) | 182.6±26.1   | 248.4±27.0    | <0.001  |
| Triglyceride level (mg/dl) | 163.6±108.7  | 243.2±167.8   | 0.018   |
| Constant score           | 41.2±17.1    | 39.3±17.8c    | 0.668   |
| Tear sizes of patients (partial/small or middle/large or massive) | 8/17/17      | 5/9/17        | 0.465   |
| Samilson–Prieto classifica- | tion of patients (normal/mild/moderate/severe) | 4/15/22/1    | 6/11/14/0 | 0.535   |

* Body mass index
b Information missing for seven patients
c Information missing for four patients

Fig. 1 Effect of cholesterol levels on the mRNA expression of MMPs and ADAMTSs mRNA expression levels of a MMP1, b MMP3, c MMP9, d MMP13, e ADAMTS4, f ADAMTS5
(P = 0.846), body mass index (P = 0.094), total cholesterol level (P = 0.202), Constant score (P = 0.549), or tear size (P = 0.516). However, significant differences were observed in the men/women ratio (P = 0.001). No significant differences in mRNA expression levels were detected between the two groups (Fig. 2).

Table 3 Clinical characteristics of low and high total triglyceride (TG) groups

|                                | Low TG group | High TG group | P-value |
|--------------------------------|--------------|---------------|---------|
| Age during surgery (years)     | 65.5 ± 8.3   | 64.2 ± 11.2   | 0.846   |
| Number of patients (men/women) | 16/17        | 34/6          | 0.001   |
| BMI* (kg/m²)                   | 24.6 ± 5.5   | 26.2 ± 4.0    | 0.094   |
| Constant score                 | 41.7 ± 17.2² | 38.9 ± 17.6¹  | 0.549   |
| Total cholesterol level (mg/dl) | 202.7 ± 38.4 | 217.1 ± 44.1  | 0.202   |
| Triglyceride level (mg/dl)     | 98.7 ± 33.3  | 278.4 ± 145.1 | < 0.001 |
| Tear sizes of patients         | 4/13/16      | 9/13/18       | 0.501   |
| (partial/small or middle/large |             |               |         |
| or massive)                    |              |               |         |
| Samilson–Prieto classification | 5/14/14/0    | 5/12/22/1     | 0.516   |
| of patients (normal/mild/      |              |               |         |
| moderate/severe)               |              |               |         |

* Body mass index
² Information missing for three patients
¹ Information missing for eleven patients

Discussion

In this study, ADAMTS5 and MMP1 expression in the synovial membrane of the glenohumeral joint was found to be significantly higher in patients with high TC levels than in those with low TC levels. In contrast, no significant differences were observed between the two TG groups. Therefore, hypercholesterolemia altered synovial MMP1 and ADAMTS5 levels in the glenohumeral joint.

![Fig. 2 Effect of triglyceride levels on the mRNA expression of MMPs and ADAMTSs](image-url)
MMP-1 is primarily produced by the synovial cells that line the joints [12]. MMP-1 has the unique ability to cleave the triple helix of collagen. Cleavage allows the chains to unwind, which makes them susceptible to further degradation by other MMPs [21]. A relationship between MMP1 expression and cholesterol levels has been identified in both in vitro and in vivo animal studies [22–24]. Cholesterol crystals stimulate MMP1 mRNA expression in human macrophages in vitro [22]. Oxidized LDL promotes MMP1 production in cultured synoviocytes derived from patients with rheumatoid arthritis [23]. Hypercholesterolemic rabbits exhibited increased MMP1 levels in the aorta [24]. In this study, MMP1 expression in the glenohumeral synovia was confirmed to significantly differ between patients with high and low TC levels, although this was not confirmed between patients with high and low TG levels. Rotator cuff MMP1 levels in synovial fluid are higher in patients with rotator tears than in healthy controls [25]. MMP1 levels in RCT patients with a massive full thickness tear were significantly higher than in patients with a partial-thickness tear and a non-massive full-thickness tear [26]. In our study, tear size did not differ between the high TC and low TC groups, suggesting the possibility that hypercholesterolemia increases MMP1 levels in the glenohumeral joint.

ADAMTS5 has emerged as a principal mediator of aggrecan loss in OA [27, 28]. ADAMTS-5 knockout mice were reportedly protected from synovitis and joint destruction [29, 30]. Some studies have focused on the relationship between ADAMTS5 and cholesterol levels. ADAMTS5 was elevated in the nucleus pulposus cells of APO-E knockout rabbits [31]. Intra-articular anti-ADAMTS5 antibody slowed down the progression of OA in a dose-dependent manner in a murine OA model with hypercholesterolemia [32]. We found that patients with hypercholesterolemia had higher expression levels of synovial ADAMTS5. A previous study reported that cartilage obtained from patients with shoulder OA had higher ADAMTS5 mRNA expression than cartilage obtained from non-OA patients [33]. Some of our present patients had OA. However, no significant difference was found in OA grade in the high and low TC groups. Therefore, our findings suggest that hypercholesterolemia might also affect ADAMTS5 expression in the glenohumeral synovium.

Several studies have reported a possible association between knee OA and TG levels, albeit that none of these associations reached statistical significance [10, 34, 35]. In our study, expression of MMPs and ADAMTSs did not differ between patients with low and high TG levels. Corroborating previous findings [10, 34, 35], our study suggests that the presence of hypertriglyceridemia has less influence on the glenohumeral joint.

Hypercholesterolemia leads to structural, inflammatory and mechanical changes in tendons, which predispose hypercholesterolemic patients to a greater risk of tendon pathology [4]. Shoulder arthritis can occur in patients with RCT, and in severe cases, the cartilage degeneration may be related to extra mechanical loading resulting from the rotator insufficiency [36]. Given that impaired mechanical loading increases MMP1 and ADAMTS5 in synovial cells [37, 38], our results might partly reflect the upregulation of mechanical stress factors due to tendinopathy. Further investigation using non-RCT patients may help reveal the direct link between synovial pathology and hypercholesterolemia.

Several limitations of this study warrant mention. First, the major limitation is that the only measure used was PCR. Protein profiling studies such as western blot and immunohistochemical analysis are needed to validate our gene expression profile results. Second, we did not include a healthy control population, although this would have been preferable as an ideal control group. Finally, any directly link in the etiology of OA with MMP1 and ADAMTS5 remains unclear.

Conclusions
Hypercholesterolemia may alter MMP1 and ADAMTS5 expression in synovium of the glenohumeral joint.

Abbreviations
OA: Osteoarthritis; ADAMTSs: A Disintegrin and Metalloprotease with Thrombospondin Motifs; MMPs: Matrix metalloproteases; RCTs: Rotator cuff tears; TC: Total cholesterol; TG: Triglyceride; JAS: Japan Atherosclerosis Society; SD: Standard deviation; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

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Authors’ contributions
KM, TK, KU, MN, RT, DI, and MS acquired the data. GI and MT analyzed and interpreted the data. All authors are responsible for the study concept and design, and contributed to writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Datasets supporting the conclusions of this article are included within the article. The raw data can be requested from the corresponding author.

Declarations
Ethics approval and consent to participate
This study was conducted with the approval of the Ethics Committee at our institution (Clinical Research Review Board of the Kitasato Institute; reference number KMEO B13-113) and abides by the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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