Synovial Fluid Ghrelin Levels in Patients With or Without Cartilage Injury Undergoing Anterior Cruciate Ligament Reconstruction

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Abstract

Objectives

The aim of our study was to measure synovial fluid levels of ghrelin in patients with ACL injury to evaluate its potential function as a diagnostic biomarker for cartilage injury.

Methods

Patients were prospectively recruited in this study, comprising a cohort of subjects that had synovial fluid samples collected. Patients' synovial fluid sampling was collected to evaluate ghrelin level. Venous blood samples were taken from all subjects to test inflammation markers IL-6, TNF- α , MMP-3, MMP-13 and also serum ghrelin level. Correlation between synovial fluid ghrelin level and inflammatory biomarkers were analyzed using spearman's correlation analysis. The best cut-off of synovial fluid ghrelin level was defined using Medcalc.

Results

Patients in the ACL reconstruction with cartilage damage had lowest synovial fluid ghrelin level (ACL reconstruction with cartilage damage group: 264.6 ± 65 pg/ml, ACL reconstruction without cartilage damage group: 466.8 ± 92.6 pg/ml, healthy controls: 471.5 ± 89.3 pg/ml, $P < 0.001$). Synovial fluid ghrelin level was negatively associated with IL-6 ($r = -0.700$, $P < 0.001$), MMP-3 ($r = -0.702$, $P < 0.001$) and MANKIN score ($r = -0.768$, $P < 0.001$). Patients with synovial fluid ghrelin level ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.

Discussion

In conclusion, synovial fluid ghrelin was a useful biomarker to predict cartilage injury for patients undergoing ACL reconstruction. Synovial fluid ghrelin was negatively correlated with IL-6, MMP-3 and MANKIN score. Synovial fluid ghrelin ≤ 333 pg/ml was a useful biomarker for cartilage damage in patients with ACL injury.

Introduction

The anterior cruciate ligament (ACL) is one of the key ligaments that help stabilize knee joint. It connects femur to your tibia. Because of the special structures of anatomy, it's most commonly torn during sports that involve sudden stops and changes in and frequently reconstructed in young, active patients. ACL injury accounts for 40% of sports injury [1]. ACL injury leads to knee instability [2], prevents patients back to normal activities and results in lower quality of life [3]. ACL injury is usually associate with the menisci injury, articular cartilage damage and subchondral or cancellous bone injury [4]. ACL reconstruction is

generally recommended if patient is athlete and wants to continue in sport, more than one ligament or the meniscus is injured, the injury causes patient's knee to buckle during everyday activities or when patient is young. A successful ACL reconstruction followed by focused rehabilitation usually would restore knee's stability and function. However, the subsequent problem after operation often occurs, which happens to 50–60% of patients receiving ACL reconstruction during follow up period [4–6].

ACL rupture leads to anterior migration of the tibia with collision of posterolateral tibial platform against the middle of the lateral femoral condyle. In most of the patients affected by the collision injury, they had a bone marrow edema [7, 8]. When collision, the articular cartilage covered by the bone marrow edema lesion suffered from significant loads, probably causing irreversible local injury and releasing proinflammatory, catabolic cytokines, and chemokines into the joint space [9–11]. This alteration in the inflammatory biomarkers at the time of ACL injury, together with its associated chondral impaction, makes a contribution to the development of osteoarthritis [12]. A cohort study of 249 individuals who had undergone primary single-bundle ACL reconstruction also showed that 39% patients develop osteoarthritis in average 7.8 years after surgery [13]. ACL reconstructions demonstrate higher prevalence of cartilage injury. Articular cartilage injury happened with ACL reconstruction probably has the most single effect on subjective outcomes during long time follow-up [14]. However, so far, cartilage damage poses a challenging diagnostic problem. In general, the diagnosis of cartilage injury is based on the radiology together with symptoms of knee pain, instability and patients' functional restrictions [15]. But this method is not sensitive enough for early stage cartilage injury and patients would thus possibly miss the best treatment options [16]. Thus, it is meaningful to search for more reliable parameters to detect early stage cartilage injury in patients with ACL injury.

Recently, synovial fluid inflammatory biomarkers have gained more significant attention since its ability to provide a window into the molecular milieu associated with various ACL injury. These biomarkers are articulation specific and could demonstrate mechanisms of the disease, such as joint inflammation and cartilage injury [17]. Several studies implied that these inflammatory markers of joint could be used to detect early cartilage metabolism after ACL injury [18]. Thus, analysis of knee joint synovial fluid biomarkers may be used as clinically relevant method to detect early biomarkers of cartilage injury.

Ghrelin is 28 amino acid hormone secreted primarily by P/D1 cells from stomach [19], which acts as a ligand for binding to the specific growth hormone secretagogue receptor [20]. Apart from the growth hormone releasing function, ghrelin has a wide range of other properties, such as influence of insulin secretion and glucose metabolism and involving in cell proliferation, regulation of gastrointestinal and immune function [21]. Ghrelin has the function in regulation of bone metabolism, promoting the proliferation of osteoblasts and differentiation of osteoblasts in mouse model and modulating bone structure [22]. In recent years, ghrelin mRNA has been detected by RT-PCR both in chondrocyte cell lines and cartilage tissues, serving as a growth factor for chondrocytes [23], which suggests the function of this novel peptide in chondrocytes as component of the growth hormone axis in cartilage metabolism. On one side, ghrelin increases cyclic adenosine monophosphate (cAMP) production in chondrocytes that is related to an augmentation of sulfated proteoglycan and hyaluronate synthesis [23]. What's more, ghrelin

was proved to increase gene expression level of chondroitin sulfate type IV, taking part in chondrocyte metabolism through promoting hypertrophy to reinforce proteoglycan synthesis [23].

Several previous publications proved that ghrelin has anti-inflammatory function through suppressing proinflammatory cytokines, e.g. interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) and interleukin-1 beta (IL-1 β) [24], which play vital roles in the ACL injury. Ghrelin reduced IL-1 β -induced expression of matrix metalloproteinases (MMPs) including MMP-3, MMP-13 in a concentration dependent manner in human chondrocytes [25], identifying a novel function of ghrelin on inhibiting the degradation of type II collagen and aggrecan.

These studies suggest that ghrelin might serve as a vital factor during the process of cartilage injury. However, there are no published studies illustrating the relationship between synovial fluid ghrelin concentrations in patients after ACL injury, with or without cartilage injury compared with healthy controls. The aim of our study was to measure synovial fluid levels of ghrelin in patients with ACL injury to evaluate its potential function as a diagnostic biomarker for cartilage injury. We hypothesized that ghrelin level is associated with cartilage injury and would be decreased in patients with ACL injury compared with healthy controls, with the lowest levels seen among patients with ACL injury together with cartilage degradation.

Patients And Method

Study patients

60 patients aged between 20–37 years old were prospectively recruited in this study, comprising a cohort of subjects that had synovial fluid samples collected. All patients were referred by Peking University Third Hospital based on the clinical examination and magnetic resonance imaging (MRI) findings.

Inclusion criteria is patients undergoing ACL reconstruction using knee arthroscopy. Exclusion criteria included patients < 18 years, patients with tuberculous, rheumatoid arthritis, gout, systematic inflammatory disease, cerebrovascular disease, liver or renal insufficiency, cardiac insufficiency, autoimmune disease, malignant tumor, or other severe systemic diseases, intra-articular injection 3 months before surgery, previous knee surgery, insufficient synovial fluid aspiration. All patients were not allowed to receive steroids or hyaluronic acid injection prior to this study. All patients undergoing knee arthroscopy who reported no pain and no history of injury to their contralateral knee were also invited with their agreement to provide a sample from the contralateral knee at the time of surgery to serve as a healthy control. The study was approved by the Ethics Committee of Peking University Third Hospital. All patients signed the written informed consent.

Synovial Fluid Collection and Storage

Patients' synovial fluid sampling was collected using the superolateral portal location before knee debridement and ACL reconstruction. We collected the maximal amount of synovial fluid that egressed. Synovial fluid samples were transferred to sterile tubes containing a protease inhibitor cocktail solution (Halt Protease Inhibitor Cocktail, EDTA-free; Pierce Biotechnology, Rockford, IL). The samples were stored on ice during transport to the laboratory where they were centrifuged to remove cells and joint debris after synovial fluid sample collection at 1350, 10 g for 10 minutes and storage at -80°C.

Laboratory methods

Venous blood samples were taken from all subjects by sterile venipuncture in the morning after an overnight fast. The separated serum was kept frozen at -80 °C prior to the investigation. Serum and synovial fluid ghrelin levels were measured by radioimmunoassay (RIA) kit (LINCO Research, St. Charles, MO, USA). The inflammation markers IL-6, TNF- α , MMP-3, MMP-13 were examined by commercially available enzyme-linked immunosorbent assays (both from IBL America, Minneapolis, MN, USA). These specific inflammatory biomarkers were chosen because they are commonly found in ACL injury and cartilage degeneration.

Definition of histopathological severity of cartilage

The cartilage samples were collected from all patients. The histopathological gradation of the severity was assessed by the histologic/histochemical grading system (MANKIN system) [26], which evaluates four parts of cartilage injury including structure, cellular abnormalities, matrix staining and tidemark integrity. 0 point means the normal cartilage and 14 points mean that the cartilage was severely injured. The MANKIN system has proven to be an excellent and reliable method to test histological scoring of cartilage changes [27].

Statistical Methods

We did all statistical analysis using SPSS 23 (IBM, Armonk, NY). Descriptive statistical analyses of baseline characteristics were conducted for all continuous variables by mean values (percentage) and categorical variables by numbers (percentage). Comparisons between patients with ACL reconstruction with cartilage damage, ACL reconstruction without cartilage damage and healthy controls were made using One-Way ANOVA with $P < 0.05$ considered as statistically significant. Correlation between synovial fluid ghrelin level and inflammatory biomarkers were analyzed using spearman's correlation analysis. The best cut-off of synovial fluid ghrelin level was defined using Medcalc.

Results

Baseline characteristics of the study groups

In total, 60 patients, all undergoing ACL construction with or without cartilage injury consented to participate in the study from July 2016 and January 2018: 25 patients had cartilage damage and 35

patients did not have cartilage damage. Baseline characteristics, such as gender, age, body mass index (BMI) and MANKIN score are summarized in Table 1.

Table 1
Patients baseline characteristics

	ACL reconstruction with cartilage damage	ACL reconstruction without cartilage damage
Number of patients	25	35
Age median, year (range)	27 (23–37)	24 (20–34)
Sex: female	10	7
Sex: male	15	28
BMI	24.9	25.8
MANKIN score	10.9±1.6	0.7±1.1

Patients ghrelin level and synovial fluid inflammatory biomarkers

Baseline ghrelin level and synovial fluid inflammatory biomarkers are summarized in Table 2. Serum ghrelin level did not reach statistical difference between ACL reconstruction with cartilage damage group and ACL reconstruction without cartilage damage group (244.6 ± 60.2 vs. 324.5 ± 73.6 pg/ml, $P = 0.323$). However, synovial fluid ghrelin level in the ACL reconstruction with cartilage damage group was lower compared with ACL reconstruction without cartilage damage group (264.6 ± 65.0 vs. 464.5 ± 92.9 pg/ml, $P = 0.019$). Patients in the ACL reconstruction with cartilage damage group had higher level of IL-6 compared with patients in the ACL reconstruction without cartilage damage group (48.5 ± 10.5 vs. 17.6 ± 6.3 pg/ml, $P = 0.021$). Patients in the ACL reconstruction with cartilage damage group had higher level of MMP-3 compared with patients in the ACL reconstruction without cartilage damage group (21.0 ± 3.8 vs. 11.1 ± 1.8 pg/ml, $P = 0.047$). The difference of TNF- α and MMP-13 did not reach statistical difference.

Table 2
Baseline ghrelin level and synovial fluid inflammatory biomarkers

	ACL reconstruction with cartilage damage	ACL reconstruction without cartilage damage	P value
Serum ghrelin level (pg/ml)	244.6±60.2	324.5±73.6	0.323
synovial fluid ghrelin level (pg/ml)	264.6±65.0	464.5±92.9	0.019
synovial fluid TNF-α (pg/ml)	12.8±3.4	13.0±3.4	0.998
synovial fluid IL-6 (pg/ml)	48.5±10.5	17.6±6.3	0.021
synovial fluid MMP-3	21.0±3.8	11.1±1.8	0.047
synovial fluid MMP-13	78.6±10.1	44.6±8.1	0.181
Patients in the ACL reconstruction with cartilage damage had lowest synovial fluid ghrelin level (ACL reconstruction with cartilage damage group: 264.6±65 pg/ml, ACL reconstruction without cartilage damage group: 466.8±92.6 pg/ml, healthy controls: 471.5±89.3 pg/ml, $P < 0.001$).			
Synovial fluid ghrelin level was negatively correlated with IL-6 ($r = -0.700$, $P < 0.001$),			
Synovial fluid ghrelin level was negatively correlated with MMP-3 ($r = -0.702$, $P < 0.001$).			
Synovial fluid ghrelin level was negatively correlated with MANKIN score ($r = -0.768$, $P < 0.001$).			
Patients with synovial fluid ghrelin level ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.			

Synovial fluid ghrelin level in ACL reconstruction with or without cartilage damage compared with healthy controls.

Patients in the ACL reconstruction with cartilage damage had lowest synovial fluid ghrelin level (ACL reconstruction with cartilage damage group: 264.6±65 pg/ml, ACL reconstruction without cartilage damage group: 466.8±92.6 pg/ml, healthy controls: 471.5±89.3 pg/ml, $P < 0.001$, Fig. 1). Synovial fluid ghrelin level was negatively correlated with IL-6 ($r = -0.700$, $P < 0.001$, Fig. 2), MMP-3 ($r = -0.702$, $P < 0.001$, Fig. 3) and MANKIN score ($r = -0.768$, $P < 0.001$, Fig. 4).

Defining the best cut-off of Synovial fluid ghrelin level as predictor of cartilage injury

We defined the best cut-off of synovial fluid ghrelin level as predictor of cartilage injury using Medcalc software. Figure 5 shows the ROC curves using the interactive dot diagram. Patients with synovial fluid ghrelin level ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.

Discussion

Our study identified a specific biomarker, synovial fluid ghrelin that differed significantly between knees with ACL constructions with cartilage injury or without cartilage injury and contralateral controls. The use of biomarkers for the purpose of the diagnosis and making treatment decision gains more attention and has become widely acknowledged in modern medicine. Most of these publications identified the use of blood samples or urine samples as biomarkers. However, it is now more widely accepted that synovial fluid describes a more accurate intra articular pathology [28]. Our study proved the same results, that synovial fluid ghrelin was significantly different between patients receiving ACL construction with cartilage injury compared with without cartilage injury. However, we could not find the difference between the two groups from serum samples. This indicates that synovial fluid ghrelin is a better biomarker to predict ACL construction with cartilage injury.

Ghrelin is a type of polypeptide hormone largely from the stomach, which was found to be the first endogenous ligand of growth hormone secretagogue receptor-1A [29]. Now, ghrelin was found to have the function to regulate growth plate chondrocyte function through the autocrine/paracrine pathways [30]. A recent study found that ghrelin plays a vital role in upregulating cartilage-specific genes on chondrocytes by activating ERK/STAT3 signaling pathway [31]. Our study was in accordance with these above published articles, indicating synovial fluid ghrelin ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.

Our study also showed the correlation of synovial fluid ghrelin with IL-6 and MMP-3. IL-6 was discovered as a cytokine that promotes B-cell differentiation and proliferation and induces antibody production, but was subsequently shown to be produced by a variety of cells including chondrocytes and synovial cells [32]. In addition, IL-6 has the function of inducing production of acute phase reactants as a pre-inflammatory cytokine [33]. In accordance with previous publications [33, 34], IL-6 was higher in the ACL construction with cartilage injury group compared with ACL construction without cartilage injury. We also proved that synovial fluid ghrelin was negatively correlated with IL-6.

MMP-3, also called proteoglycanase, is an important enzyme in the process of cartilage destruction [35], since it has the function of decomposing vital molecular structures of the cartilage matrix, including glycan, type IX collagen, fibronectin and is also taking part in the activation of other MMPs. Many cells, including synovial fibroblasts, produce MMP-3. Several studies have shown that a positive correlation exists between the intensity of MMP-3 staining and matrix degradation in cartilage tissues [36]. The MMP-3 concentration in the synovial fluid of patients with osteoarthritis was associated with the disease progresses, proving that MMP-3 is an biomarker of cartilage destruction [37]. Our study proved MMP-3 was higher in the ACL construction with cartilage injury group compared with ACL construction without cartilage injury. We also proved that synovial fluid ghrelin was negatively correlated with MMP-3.

Our study also proved that synovial fluid ghrelin was negatively correlated with MANKIN score, indicating its function as a prognostic biomarker.

Our study has several limitations. Our study is an prospective observational study. Our sample size is not large enough. The results from our study still needs to be validated in a large scale clinical trial. However, our study is the first to take synovial fluid ghrelin into consideration for patients receiving ACL reconstruction with or without cartilage injury. It worth further studies in the field and more interventions are needed to help patients treat the disease. We also identified the best cut-off point of synovial fluid ghrelin as biomarkers for cartilage injury, which would also needs more validation studies.

Conclusion

In conclusion, synovial fluid ghrelin was a useful biomarker to predict cartilage injury for patients undergoing ACL reconstruction. Synovial fluid ghrelin was negatively correlated with IL-6, MMP-3 and MANKIN score. Synovial fluid ghrelin ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.

Declarations

Ethics approval and consent to participate

The studies involving human subjects were reviewed and approved by the Medical Ethics Review Committee of Peking University Third Hospital. All patients who participated in this study provided written informed consent during the study.

Competing interests

Not applicable.

Consent for publication

Not applicable.

Authors' contributions

Jiakuo Yu designed the study. Zimu Mao searched articles and collected data, Zeyi Chen did statistical analysis, Zimu Mao wrote the manuscript, all the other authors read and approved the final form of the manuscript.

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Declarations

All data in our study would be available upon request.

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Figures

Figure 1

Synovial fluid ghrelin levels in ACL reconstruction with or without cartilage damage compared with healthy controls. Patients in the ACL reconstruction with cartilage damage had lowest synovial fluid ghrelin level (ACL reconstruction with cartilage damage group: 264.6 ± 65 pg/ml, ACL reconstruction without cartilage damage group: 466.8 ± 92.6 pg/ml, healthy controls: 471.5 ± 89.3 pg/ml, $P < 0.001$).

Figure 2

Correlation of synovial fluid ghrelin with IL-6. Synovial fluid ghrelin level was negatively correlated with IL-6 ($r = -0.700$, $P < 0.001$),

Figure 3

Correlation of synovial fluid ghrelin with MMP-3. Synovial fluid ghrelin level was negatively correlated with MMP-3 ($r=-0.702, P < 0.001$).

Figure 4

Correlation of synovial fluid ghrelin with MANKIN score. Synovial fluid ghrelin level was negatively correlated with MANKIN score ($r=-0.768$, $P < 0.001$).

Figure 5

The ROC curves using the interactive dot diagram. Patients with synovial fluid ghrelin level ≤ 333 pg/ml had higher chance of cartilage injury with specificity of 91.7% and sensitivity of 87.5%.