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Original Article

Correlation of MRI T2-star mapping and the expression of γ-glutamyl carboxylase in degenerative knee cartilage

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Abstract: Objective: The study objective was to investigate the correlation of MRI T2-star mapping and the expression of γ-glutamyl carboxylase (GGCX) in degenerative knee cartilage. Methods: In this study, 20 female patients with mild, moderate, or severe osteoarthritis (OA) were selected as the study subjects, and 20 healthy women were used as the control group. Using a GE 3.0T magnetic resonances canner, a conventional scan of the knee was first performed (sagittal, coronal T1WI and T2WI, axial T2WI), followed by T2-star mapping and diffusion-weighted imaging. The cartilage specimens in the NMR region of interest were obtained, and the expression level of GGCX in the cartilage was detected by immunohistochemistry and Western blot assays. Results: The averages of the T2-star values of different segments of the articular cartilage with mild, moderate, and severe knee OA were significantly higher than those in the control group, with statistically significant differences ($P<0.05$), and they were negatively correlated with the GGCX content in the cartilage. Conclusions: MRI T2-star mapping and the expression of GGCX in degenerative knee cartilage were found to be closely related. This correlation allows for early diagnoses prior to morphological changes in the knee cartilage as well as early intervention measures to improve treatment outcomes.

Keywords: Knee osteoarthritis, articular cartilage, T2-star mapping, γ-glutamyl carboxylase

Introduction

Osteoarthritis (OA) is a degenerative joint disease characterized by articular cartilage injury and hyperostosis featuring degenerative damage in the articular cartilage [1]. The biochemical composition and structure of the articular cartilage are already altered in patients with early-stage OA who do not yet exhibit obvious clinical symptoms. Magnetic resonance imaging (MRI) T2-star mapping clearly shows the shape and the signal changes of the articular cartilage, allowing one to obtain the T2 relaxation time for the articular cartilage and quantitatively analyze its biochemical composition and structure. T2-star mapping facilitates improved quantitative analysis over other magnetic resonance methods and is more sensitive to the detection of damaged and degenerating articular cartilage [2]. Most of the current research in the field focuses on the correlation between the quantitative analysis of the articular cartilage components by MRI T2-star mapping and the pathology and morphology of the cartilage, and relevant studies at the molecular level are rarely reported.

A previous study found that γ-glutamy lcarboxylase (GGCX) is expressed in the articular cartilage of both normal knees and knees with primary osteoarthritis and is predominantly located in the cytoplasm of the chondrocytes [3]. The expression of GGCX is significantly lower in primary knee osteoarthritis versus normal cartilage, and severe cartilage degeneration is proportionally associated with decreased GGCX expression [4]. In the present study, the correlation between the MRI T2* value and the GGCX content in the cartilage of OA patients was investigated. This correlation allows for the

Table 1. General information between OA patients and healthy control

Group	Healthy control (n=20)	OA group (n=60)		
		Mild (n=20)	Moderate (n=20)	Severe (n=20)
Age	56.6±3.2	57.1±4.0	56.8±3.7	57.5±3.3
BMI	20.3±1.6	20.7±2.1	21.2±1.8	20.9±2.2

early diagnosis of cartilage degeneration prior to the detection of morphological changes, which enables early intervention measures to improve treatment outcomes.

Materials and methods

Clinical data

The cartilage tissues were voluntarilydonated-by 60 patients who underwent total knee arthroplasty or joint debridement due to primary knee OA in the Orthopedics Department at Ningxia People's Hospital from September 2013 to September 2015. A total of 60 female patients with clinically diagnosed mild, moderate, or severe osteoarthritis (OA) (20 cases each) were designated the OA group. In addition, normal cartilage was collected from patients who underwent arthroscopic surgery for the removal of free cartilage fragments in the knee or amputation after trauma during the same time period (20 cases); these voluntary donations of normal articular cartilage served as the control group. There were no significant differences in age, sex, or body mass index (BMI) between the OA patients and healthy controls (P>0.05) (**Table 1**).

Inclusion criteria

The healthy volunteers were selected based on the following criteria: ① A WOMAC scoreof 10 or lesson the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) in the USA [5], and ② the height, weight, and body mass index (BMI) of the normal volunteers excluded the impact of underweight or obesity on the data. The OA study subjects met the diagnostic criteria of the 2007 Osteoarthritis Diagnosis and Treatment Guidelines. The knee cartilagede generation in the OA patients was graded according to the Kellgren-Lawrence assessing criteria [6] formild, moderate, and severe OA.

Exclusion criteria

The control group excluded individuals under 18 years of age, those who had a history of knee-related diseases, those with trauma or abnormalities upon physical examination, those who had received analgesic anti-inflammatory or palliative drugs within one month, athletes, hardphysical laborers, those with a WOMAC score greater than 10, and people with a BMI greater than 18.5-23.9 kg/m^2. The early osteoarthritis group excluded individuals with obvious manifestations of osteoarthritis via X-ray or CT/MRI.

Magnetic resonance imaging

First, a GE SignaHDx3.0T (GE Healthcare, USA) magnetic resonances canner with an 8-channelkneecoilwas used for conventional knee scanning (sagittal, coronal T1WI and T2WI, axial T2WI), followed by T2-star mapping imaging and diffusion-weighted imaging. The resulting images were post-processed at a GEAW4.5 workstation. The NM Rregion of interest was manually selected in the medial tibialcondyle surface of the normal and the osteoarthritis groups, with a scope of approximately 1 cm^2. The T2* values were obtained by averaging at least 3 parallel measurements. Images with severe sub-region TV motion artifacts (t>200 ms) were excluded from the study.

GGCX protein detection

The obtained cartilage tissue was divided in half: the first half was placed in 10% neutral-carboxylic acid for GGCX protein detection by immunohistochemistry, while the second half was stored in -80°C liquid nitrogen immediately after collection for GGCX protein detection by Western blotting. Immunohistochemistry was performed on cartilage tissues embedded in paraffin. Immunostaining was performed using mouse anti-human GGCX antibody (SANTA, Carpinteria, CA) as a primary antibody (overnight at 4°C), followed by incubation with Horse Radish Peroxidase (HRP)-conjugated goat anti-mouse IgG (30 minutes at room temperature; SANTA).

Statistical analysis

The SPSS19.0 software package was used for data entry and statistical analysis. The articular cartilage T2* values were represented as the

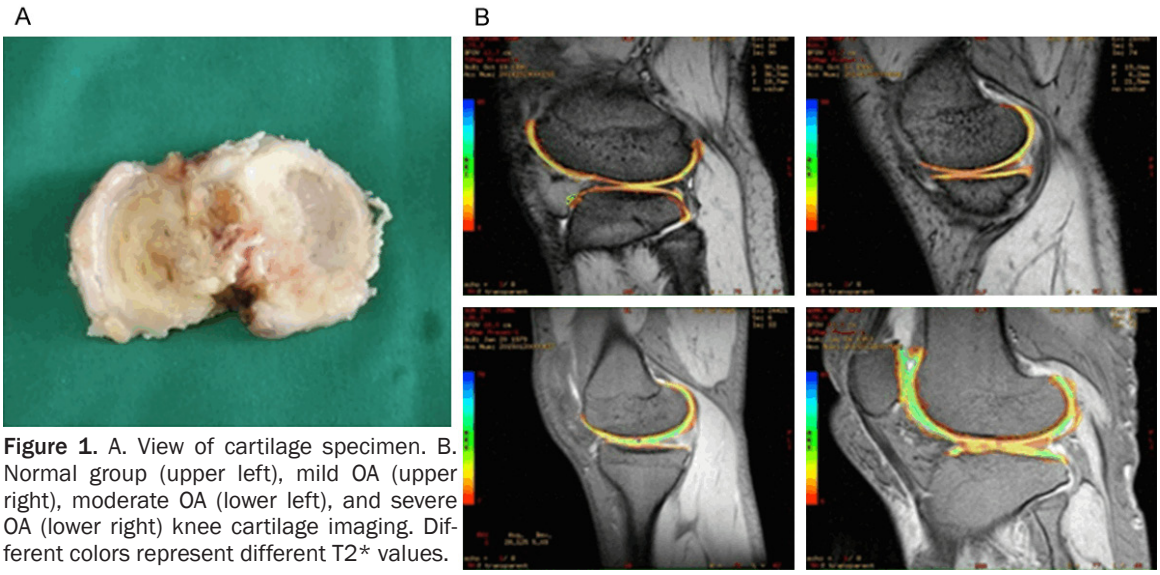

Figure 1. A. View of cartilage specimen. B. Normal group (upper left), mild OA (upper right), moderate OA (lower left), and severe OA (lower right) knee cartilage imaging. Different colors represent different T2* values.

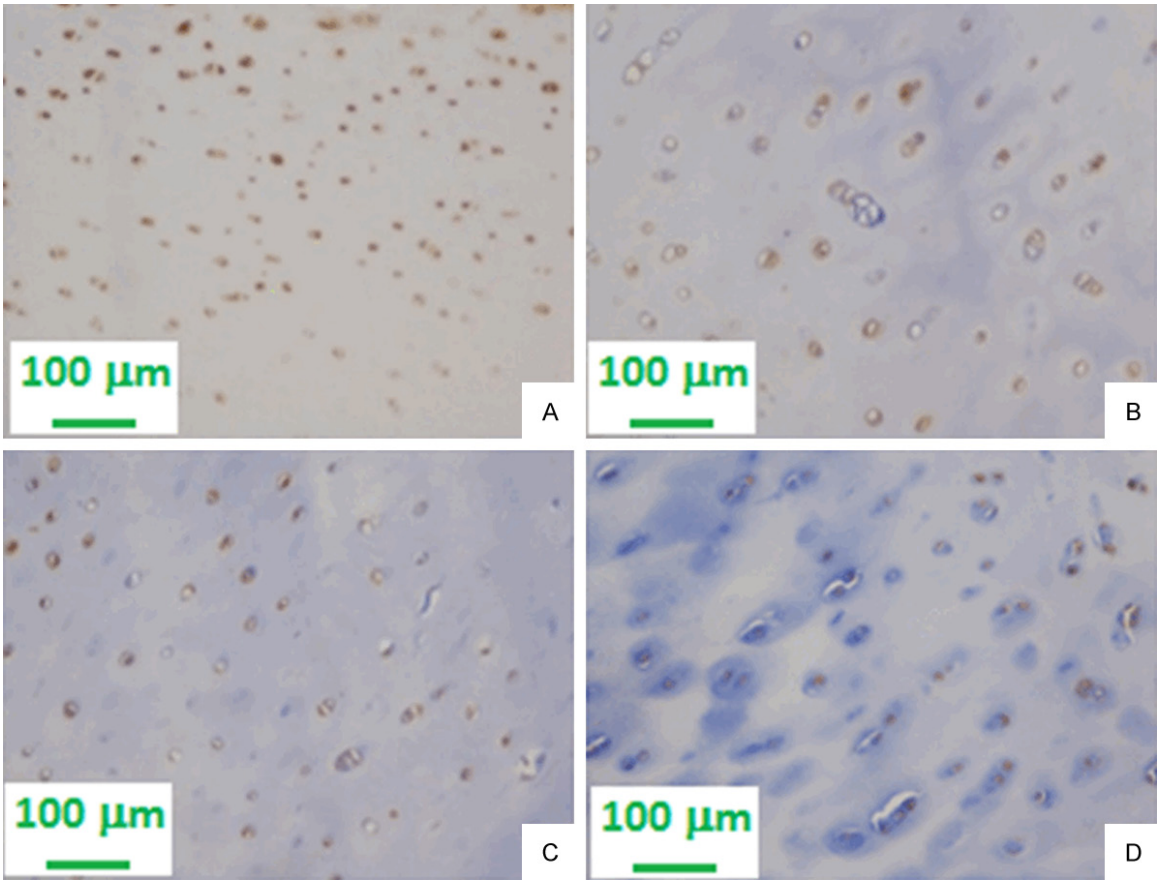

Figure 2. GGCX expression in the OA cartilage was detected by immunohistochemistry. A-D. Representthenormal, mild, moderate, and severe OA groups, respectively (×100).

mean ± standard deviation ($\bar{x}\pm s$). Acorrelation analysis was performed using Pearson correlation; $P<0.05$ was considered to be statistically significant.

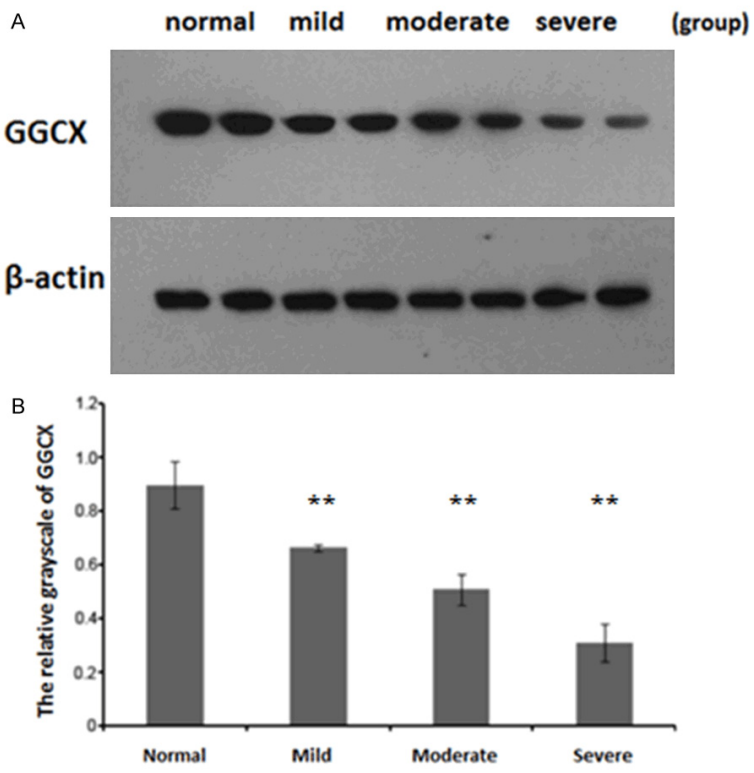

Figure 3. A. The expression of GGCX in OA cartilage was detected by Western blot. B. And then the relative grayscale values of GGCX in each group were shown. **Compared with normal group, *P<0.01*.

Results

Observation of the cartilage specimens

The articular cartilage in the OA group showed a rough and dull yellow or pale yellow surface with fissures, cracks, erosion, and ulcers of different sizes; in some areas, particularly in weight-bearing areas, the subchondral bone was exposed due to full-thickness damage of the cartilage (**Figure 1A** and **1B**).

Detection of GGCX expression in the OA cartilage by immunohistochemistry

GGCX was expressed in the articular cartilage of both normal (**Figure 2A**) and primary osteoarthritic knees (**Figure 2B-D**) and was primarily located in the cytoplasm of the chondrocytes, as indicated by uneven yellow or brown yellow positive staining and a scattered distribution (**Figure 2**). Darkstaining was found in normal articular cartilage, whereas light staining was found in osteoarthritic cartilage (**Figure 2B-D**). The average integrated opticaldensities (IODs) of GGCX in the articular cartilage of the primary osteoarthritic and normal knee joints were 1.344±0.126 and 0.592±0.261, respectively, and the difference was statistically significant (P<0.05).

Detection of the expression of GGCX in OA cartilage by western blot

The IOD value of each gelb and was determined, and the gray scale ratio of the GGCX band to the internal reference β-actin signified the relative expression level of the GGCX protein (**Figure 3**). GGCX expression was found in the normal group and in all the OA groups. The relative gray scale values of GGCX were 0.315± 0.212 for the OA groups and 0.937±0.051 for the normal group. The GGCX content of the OA groups was significantly lower than that of the normal group, and the difference between the two groups was statistically significant (P<0.01).

Correlations between the T2 values, GGCX expression, and OA severity*

The normal articular cartilage T2* values ranged from 11.733-31.886 ms, with an average of 21.74±3.82 ms; the mild OA articular cartilage T2* values ranged from 16.869-42.260 ms, with an average of 27.55±5.48 ms; the moderate OA articular cartilage T2* values ranged from 20.284-57.531 ms, with an average of 35.01±8.83 ms; the severe OA articular cartilage T2* values ranged from 21.753-94.064 ms, with an average of 41.14±14.20 ms. The averages of the T2* values of different segments of the articular cartilage with mild, moderate, and severe knee OA were significantly higher than those in the control group, with statistically significant differences (P<0.05), and they were negatively correlated with the GGCX content in the cartilage (**Figure 4A** and **4B**). Both the immunohistochemistry and Western blot results showed that the more severe the cartilage degeneration was, the lower the expression of GGCX was, and the differences were statistically significant (P<0.05). This observation suggests that the expression of GGCX exhibited a gradual downward trend

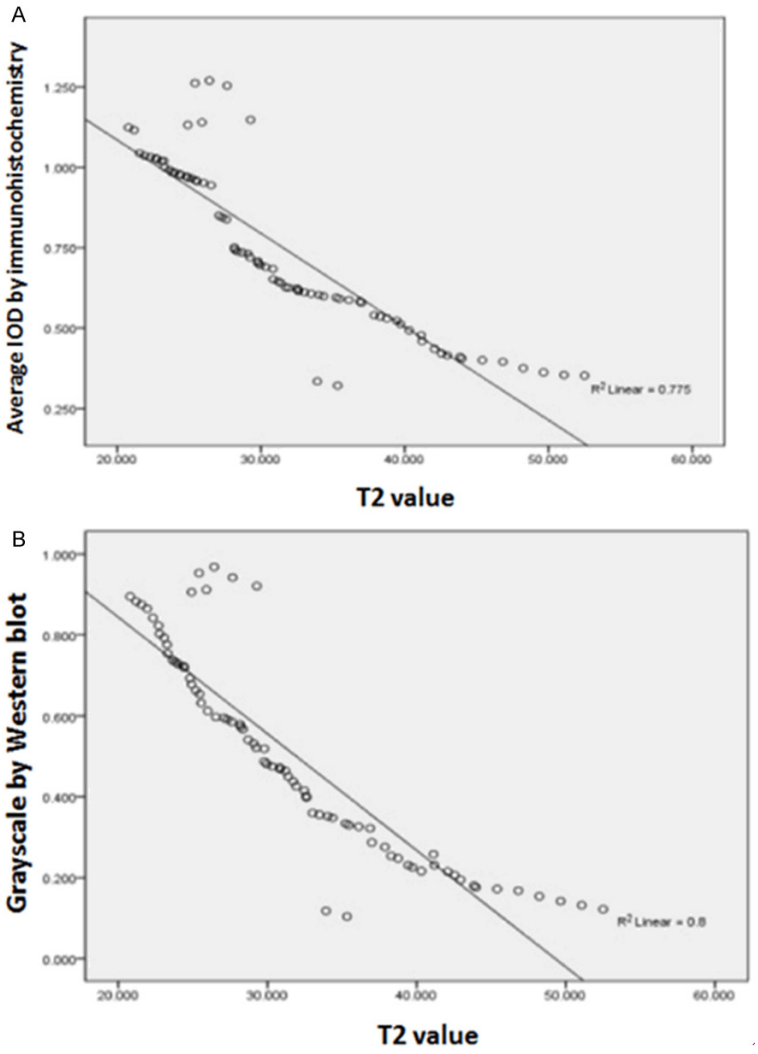

Figure 4. A, B. Correlations between the T2* values and GGCX expression (by immunohistochemistry and western blot, respectively) were analyzed using Pearson correlation.

with the increasing severity of articular cartilage degeneration in osteoarthritis; thus, the severity of osteoarthritis was related to GGCX expression.

Discussion

The significance of GGCX in knee OA

Calciumde position plays a very important role in the occurrence and development of osteoarthritis [7]. MatrixGLA protein (MGP) is a key enzyme that participates in the carboxylation process of the important inhibitory protein GGCX during the formation of calcium crystals [8], which affects the formation of calcium deposition. In the articular cartilage of knee OA patients, only uncarboxylated MGP exists, whereas, in the cartilage of the normal knee, carboxylated MGP is present [9]. The expression of GGCX was significantly lower in the primary OA knee cartilage than the normal knee cartilage, and increasingly severe cartilage degeneration was associated with progressively lower GGCX expression [4].

The value of T2 and T2-star mapping for the evaluation of articular cartilage degeneration*

T2-star imaging (T2*) applies a multi-planar multi-echogradient echo for data collection to yield T2* contrast colorscaleor gray scale images. The resulting T2* values reflect the spin-spin relaxation between adjacent protons in the tissue, as well as the transverse relaxation due to the phase shift caused by the in homogeneity of the magnetic field, thus, the measurements of T2* are smaller than the actual T2 values [10]. For the quantitative analysis of cartilage, T2-star mapping candisplay changes in the internal biochemical composition and microstructure of early OA articular cartilage, providing objective and quantitative indicators to monitor disease progression and guide clinicaltherapy [2]. In this study, we found that the articular cartilage T2* values increased with the severity of the lesions and were negatively correlated with the GGCX content in the cartilage. Thus, T2-star imaging allows for early intervention measures to be taken to improve disease treatment efficacy, which is consistent with the findings of other studies [11, 12]. Consequently, we believe that MRI T2-star mapping for the quantitative analysis of cartilage promotes the early diagnosis of OA. Conventional X-ray examination and MRI sequen-

tial scanning of patient scan only reveal advanced stage OA with joint space narrowing and joint deformities; early OA cannot be detected. MRI T2-star mapping can reflect changes in the internal composition of the cartilage via quantitative analysis of the internal components of the articular cartilage tissue, which aids the detection of early lesions [13]. Early diagnosis and treatment of OA patient scan slow the natural progression of OA and prevent further OA development.

Limitations and deficiencies of T2-star mapping technology

In the present study, the magic angle effect may have affected the articular cartilage T2* values. The magic angle effect is caused by the distribution of water matching the arrangement of collagen fibers, which leads to diverse arrangements and distributions of water molecules in different cartilage layers, resulting in a stable magnetization vector angle. Due to the curved shape of articular cartilage, when the angle between the direction of the cartilage collagen fiber and the main magnetic field is approximately 55°, the T2* value may be elevated [14, 15]. In addition, age, gender, and BMI also had some influence on the T2* results [16]. All the subjects in this study were female, so the influence of gender on the experimental results can be eliminated. Under weight and obese individuals were excluded from the study; therefore, BMI did not affect the experimental results. Future studies should involve increasing the sample size with practical categories based on age and gender. This study focused on the clinical application of MRI T2-star mapping for the quantitative analysis of cartilage for OA diagnosis. Due to time constraints, a relatively small number of healthy volunteers and clinical cases were used in the study. In future research, we will expand the sample size and develop a convenient and reliable screening method for the early diagnosis of knee OA in patients with degenerative cartilage.

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Disclosure of conflict of interest

None.

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