Glycosaminoglycan measured from synovial fluid serves as a useful indicator for progression of Osteoarthritis and complements Kellgren–Lawrence Score

Priya Kulkarni, Shantanu Deshpande, Soumya Koppikar, Sanjay Patil, Dhanashri Ingale, Abhay Harsulkar

Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth University, Pune-Satara Road, Pune 411043, India
Department of Orthopaedics, Bharati Hospital, Pune-Satara Road, Pune 411043, India

A B S T R A C T

Background: Plain radiography is the first choice for diagnosis and monitoring of knee-osteoarthritis (OA) while, Kellgren–Lawrence score (KL) is most widely used to grade OA severity. However, incompetency for reproducibility of joint space measurement in longitudinal assessment and non-linearity of KL-score system, limits radiography-based early diagnosis of the disease. Glycosaminoglycan (GAG) is direct cartilage-degradation product, which can be measured biochemically. We strived to correlate KL-score and GAG from OA patients to compliment KL-system.

Methods: We obtained 34 synovial-fluid (SF) samples from 28 OA patients (few bilateral) with different disease severity using arthrocentesis. All patients were categorised using radiographic KL-score-system. SFs were further analysed for GAG estimation using 1,2-dimethylmethylene blue (DMMB) assay.

Results: A substantial increase in GAG was noted in KL-grade-II and III, comparing grade-I patients, indicating amplified cartilage-degradation. KL-grade-IV patients revealed further rise in GAG reflecting more cartilage-loss. Another category of grade-IV patients with lower GAG were also detected, indicating close to total cartilage-loss.

Conclusions: Accurate diagnosis of cartilage-loss remains a challenge with OA due to limitations of KL-system; thus no target intervention is available to arrest active cartilage-loss. We propose, GAG-estimation in OA patients, characterizes accurate biochemical depiction of cartilage degeneration.

General Significance: Radiology often fails to reveal an accurate cartilage loss, associated with OA. GAG levels from the SFs of OA patients’ serve as a useful marker, which parallels cartilage degeneration and strengthens radiographic grading system, ultimately

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords: Glycosaminoglycan, Joint-space-narrowing, Kellgren-Lawrence score, Knee-osteoarthritis, Radiography, Synovial-fluid

1. Introduction

Cartilage is considered as an engineering marvel that handles remarkable pressure on weight bearing joints. Extracellular matrix and aggrecan, a water-laden proteoglycan, provide cartilage with a great tensile strength, stiffness and resistance for deformation. Progressive degeneration of articular cartilage is a hallmark of osteoarthritis (OA), which results in pain and loss of function. OA is expected to get worsen as we experience a global rise in obesity and associated knee injuries. Cartilage degradation is a combined outcome of ineffective physical force management and molecular damage. Disrupted cartilage metabolism involves depolymerizing enzymes like metalloproteinase, which releases glycosaminoglycan (GAG) [1,2].

At present, plain radiography remains a priority of orthopaedic clinicians to monitor and assess OA progression [3] whereas Kellgren–Lawrence Score (KL) is the most widely used radiographic metric. This system is based upon radiographic features like Joint Space Narrowing (JSN) and osteophytes formation. Easy access, cheap cost, short imaging time and less discomfort to patients are common advantages associated with radiography. However, scope of plain radiography is limited due to confines such as, lack of reproducibility of joint space measurement in longitudinal assessment, joint positioning, non-linearity in KL grades and little information about the rate of cartilage degeneration [4].

It is well understood that a variety of matrix molecules and their degradation products are released by degrading cartilage, which can
be measured biochemically [5]. Negatively charged GAG chains in aggrecan, serve a vital function of providing tensile strength to collagen fibrillar network. GAG levels in synovial fluid (SF) would therefore reflect cartilage degenerative changes associated with OA [2].

In the present communication, we attempted to correlate GAG and KL grades of OA patients with an objective to complement the limitations of KL-score system. To evaluate this hypothesis, we enrolled 28 OA patients with different disease severity and obtained 34 SF samples, including a few bilateral samples. These SFs underwent GAG analysis and obtained GAG values were further compared with patient’s KL score to establish a correlation between clinical parameter and cartilage degeneration.

2. Materials and methods

2.1. Patient and KL-score assessment

For the present study, we recruited 28 patients with varied OA severity. The disease diagnosis was performed by clinical assessment (knee pain for at least six months and on the majority of days during the preceding month) and radiology. Typical antero-posterior (AP) or lateral view X-ray of affected knee joint (standing) was obtained and graded for KL-score. The radiographic features like JSN, presence of osteophytes and sub-chondral sclerosis were considered while grading. The characteristics for each KL-grade can be summarized as, grade I — minimal OA, with definite osteophytes but unimpaired joint space, grade III — moderate OA, with osteophytes and moderate diminution of joint space whereas grade IV — severe OA, with greatly impaired joint space and sclerosis of subchondral bone [6].

2.2. Evaluation of GAG

SFs aspiration of the enrolled OA patients, who had a knee effusion, was performed under strict aseptic precautions. The affected knee was cleaned; draped and arthrocentesis was carried out using 18 gauge needle and 10-cc sterile syringe. Single needle prick method was adopted to avoid contamination; in the first step skin was punctured which was followed by the puncture of synovial capsule. Enrolled patients were briefed about the aim and objectives of this study and voluntary consent was obtained for the participation.

2.2. Collection of SFs

SFs aspiration of the enrolled OA patients, who had a knee effusion, was performed under strict aseptic precautions. The affected knee was cleaned; draped and arthrocentesis was carried out using 18 gauge needle and 10-cc sterile syringe. Single needle prick method was adopted to avoid contamination; in the first step skin was punctured which was followed by the puncture of synovial capsule. Enrolled patients were briefed about the aim and objectives of this study and voluntary consent was obtained for the participation.

2.3. Evaluation of GAG

The collected SFs were further analysed for their GAG estimation. GAG levels were measured by a spectrophotometric dye binding assay, using 1,2-dimethylmethylene blue (DMMB) with chondroitin sulphate as standard [7]. The levels were expressed as microgram equivalents of chondroitin sulphate per ml SF.

All the protocols were approved by the Institutional Ethical Committee, constituted for this purpose (BVDU/MC/56).

3. Statistical analysis

The collected data was statistically analysed using two independent samples t-test. The severity of cartilage degradation, in terms of GAG value, was compared among radiographic KL grades.

Inter-grade comparison (grade I to IV) of KL-score was performed with their respective GAG values using SAS University software (Edition 1.0). After many experiments, a p value less than 0.05 was considered as an indicator of significant difference.

4. Results

KL grade I (N = 6) showed high statistically significant difference in GAG values when compared to KL grade II (N = 7), grade III (N = 5) and grade IV patients (Table 1). However, difference in GAG remained non-significant in between KL grade II and grade III (p = 0.6395). Both the categories represent moderate to severe JSN, indicating noteworthy cartilage degradation. GAG estimation from KL grade IV also remained remarkably higher when compared to grade II and grade III (p = 0.0001, p = 0.0001 respectively). A graphical presentation of GAG estimation from all the studied patients is shown in Fig. 1.

![Fig. 1. A graphical presentation of GAG estimation in enrolled OA patients.](image)

The statistical data analysis of obtained samples is summarized in Table 2.

5. Discussion

Knee pain is shown to have a poor correlation with cartilage degeneration [4,8]. Thus, the active cartilage degeneration phase (CDP) is
exclusive for both, patient and physician. OA therefore, not only remains asymptomatic for quite a long time; but it is also difficult to predict the stage at which cartilage degradation begins and reaches the peak.

The present data revealed high GAG estimation in all SFs than its documented normal value [9], which were reflective of loss in aggrecan and thus the damaged cartilage [10]. GAG values of KL grade I patients, however found the lowest among all KL-grades, as an indicator of early OA. On the other hand, high GAG in all grade II patients was complementing the KL-score by radiographically detectable OA. The same trend continued with all KL-grade III patients and remained comparable with grade II.

Based on the GAG estimation, grade IV patients, however, were divisible into two categories. First category (patient P18 to P21) revealed a very high GAG, suggesting a peak of active cartilage degeneration process. It explained a transition from grade III to grade IV i.e. towards terminal stage of the disease. On the other hand, patients from second category (P22 to P28) were found with significantly low GAG levels. Cartilage in these patients was completely worn-out extending a profound loss of joint space, as observed from the radiographs. Interestingly, we found the similar research observations reported by Waluka et al, where a possibility of ‘floor effect’ was predicted in end-stage OA patients (KL grade IV) because of a very little leftover cartilage or its absolute loss [11]. We strongly believe, the category II with lower GAG, represented the same ‘floor effect’ as mentioned above.

Cartilage remodelling is a continuous event pivoted by a delicate balance between anabolic and catabolic process. A marginal shift towards catabolism gradually leads to cartilage loss and further the disease development, which remains asymptomatic for quite a long time. It is suggested that cartilage degeneration process in OA is not linear and rate of OA progression calculated by joint space width (JSW) widely varies from 0.06 mm/year to 0.6 mm/year. Based upon MRI imaging of 123 OA subjects, Waluka et al had estimated tibial cartilage volume loss at the rate of 5% per year [11]. Thus, it takes 1–2 years or even longer to detect the progression of damage being visible on radiographs because of its insensitive reflection of disease process [5]. By the time clinical characteristics features like joint pain, stiffness and dysfunction are diagnosed (evaluated by the clinical assessment in the form of limited range of motion and instability) a significant cartilage loss has already resulted. Since cartilage loss cannot be detected earlier, it has not been a therapeutic target so far.

Williams et al. 2004 has emphasised a need for further research to unravel biochemical changes during OA progression, this is to complement delayed-gadolinium-enhanced MRI of cartilage (dGEMRIC), an emerging non-invasive technique for GAG estimation [12]. The present study was completely attributed to scrutinize GAG values ranging from early OA to the terminal stage of the disease; thus, could be beneficial to reveal an insight of biochemical picture of cartilage degeneration in OA [12].

Although, conventionally OA is considered as a wear and tear disease and one may believe that exercise will not be helpful for its management or even worsen the situation. On the contrary, physical exercise has been shown to have protective effect against cartilage-loss in OA-animal model [13]. Further, Williams et al. 2004 has found higher GAG contents in knee-cartilage of professional dancers and those who exercise regularly. Moreover, moderate physical activity (like running) plays a beneficial role along with nutritional supplements in the OA patients with initial low GAG estimation [12]. This study is an excellent example for underscoring the importance of GAG monitoring in OA and its utility in effective disease management. We are aware that limited number of SFs in each KL grade is a constraint of the present study due to invasive method of sample collection. However, based on the current data, GAG level proved to be a useful marker, being a true indicator of cartilage loss within the knee joint. GAG estimation from SF of OA patient strengthens KL grading system, which will ultimately help clinicians to prescribe an effective therapy.

To conclude, KL-score system often fails to reveal a transition from one grade to next, unless there is a substantial cartilage loss, which is an irreversible process; thus, revealing a dichotomy between the active CDP and KL score. As this crucial gap remains un-attempted in the present scenario, arresting progressive cartilage loss is not yet a therapeutic target. Here, we attempted to evaluate a correlation between KL-score system and GAG. Although, the invasive nature may limit its disease prognostic value, being a direct degenerative product of cartilage, GAG represents a true biochemical picture of cartilage degeneration.

Patient consent

Written informed consent was obtained from the patients for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Conflict of interest

The authors have no competing interests.

Funding source

The study was funded by institutional support at Interactive Research School for Health Affairs (IRSHA).

Acknowledgement

We acknowledge Prof. Ulhas Bapat, MA, LLB, DEPS, MTS (London), Hon. Member of Cambridge Academy of English [Cambridge], St. Clare’s [Oxford], Pune, India, for his English language help.

References

[1] M. Karsdal, S. Madsen, C. Christiansen, K. Henriksen, A. Fosang, B. Sondergaard, Cartilage degradation is fully reversible in the presence of aggrecanase but not matrix metalloproteinase activity, Arthritis Res. Ther. 10 (2008) (R56).
[2] A. Poole, M. Kobayashi, T. Yasuda, S. Laverty, F. Mwale, T. Kojima, et al., Type II collagen degradation and its regulation in articular cartilage in osteoarthritis, Ann. Rheum. Dis. 61 (1178-8) (2002).
[3] P. Enrani, J. Katz, C. Kessler, W. Reichmann, E. Wright, T. McAlindon, et al., Joint space narrowing and Kellgren–Lawrence progression in knee osteoarthritis: an analytic literature synthesis, Osteoarthr. Cartil. 16 (8) (2008) (873-82).
[4] A. Guermazi, F.W. Roemer, D. Burstein, D. Hayashi, Why radiography should no longer be considered a surrogate outcome measure for longitudinal assessment of cartilage in knee osteoarthritis, Arthritis Res. Ther. 13 (2011) 247.
[5] M. Reijnman, J. Hazes, B. Serra-Zeinstra, B. Koes, S. Christgau, C. Christiansen, et al., A new marker for osteoarthritis: cross-sectional and longitudinal approach, Arthritis Rheumat. 50 (8) (2004) 2471–2478.
[6] T. Link, L. Steinbach, S. Ghosh, M. Ries, Y. Lu, N. Lane, S. Majumdar, Osteoarthritis: MR imaging findings in different stages of disease and correlation with clinical findings, Radiology 226 (2) (2003) 373–381.
[7] V. Sumantran, A. Joshi, S. Roodiel, W.D. KoppikarS, B. Patwardhan, et al., Antiarthritic activity of a standardized, multiherb, ayurvedic formulation containing Boswellia serrata: in vitro studies on knee cartilage from osteoarthritis patients, Phytother. Res. 25 (2011) 1375–1380.

Table 2

| KL grades | p value |
|-----------|---------|
| I and II  | 0.0001*** |
| I and III | 0.0001*** |
| I and IV  | 0.0001*** |
| II and III| 0.6395   |
| II and IV | 0.0001*** |
| III and IV| 0.0001*** |

*** Statistical significance at 1%.
[8] P. Paradowski, Osteoarthritis of the knee: assessing the disease, Health Care Curr. Rev. 2 (2014) 2.
[9] S.M.G. Mattiello-Rosa, P.F.A. CintraNeto, G.E.G. Lima, K.N.Z. Pinto, M. Cohen, E.R. Pimentel, Glycosaminoglycan loss from cartilage after anterior cruciate ligament rupture: influence of time since rupture and chondral injury, Rev. Bras. Fis. 12 (1) (2008) 64–69.
[10] N. Ishiguro, T. Kojima, A. Robin Poole, Mechanism of cartilage destruction in osteoarthritis, Nagoya J. Med. Sci. 65 (2002) 73–84.
[11] A. Wluka, S. Stuckey, J. Snaddon, F. Cicuttini, The determinants of change in tibial cartilage volume in osteoarthritic knees, Arthritis Rheum. 46 (8) (2002) 2065–2072.
[12] A. Williams, A. Gillis, C. McKenzie, B. Po, L. Sharma, L. Micheli, et al., Glycosaminoglycan distribution in cartilage as determined by Delayed Gadolinium-Enhanced MRI of Cartilage (dGEMRIC): potential clinical applications, Am. J. Roentgenol. 182 (2004) 167–172.
[13] E.M. Roos, L. Dahlberg, Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage: a four-month, randomized, controlled trial in patients at risk of osteoarthritis, Arthritis Rheum. 52 (11) (2005) 3507–3514.