BBA Clinical 6 (2016) 1–4

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin

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

Priya Kulkarni [a], Shantanu Deshpande [b], Soumya Koppikar [a], Sanjay Patil [b], Dhanashri Ingale [a], Abhay Harsulkar [a,*]

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

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 10 May 2016
Accepted 10 May 2016
Available online 12 May 2016

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

ABSTRACT

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 arthrocetesis. 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 strengthen radiographic grading system, ultimately

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

* Corresponding author.
E-mail addresses: pgkulkarni2610@googlemail.com (P. Kulkarni), desh75@googlemail.com (S. Deshpande), soumya.koppikar@gmail.com (S. Koppikar), sanjaypatil67@hotmail.com (S. Patil), ingaledhanashri91@gmail.com (D. Ingale), aharsulkar@yahoo.com (A. Harsulkar).

http://dx.doi.org/10.1016/j.bbacli.2016.05.002

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 anterio-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 — doubtful OA with presence of minor osteophytes of doubtful importance, grade II — 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. 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.

Table 1
Estimated GAG values and KL-scores of studied patients.

Sr. no	Patient no.	KL score	GAG
1	P1	I	0.8
2	P2	I	39.2
3	P3	I	3.8
4	P4	I	9.2
5	P5	I	20.9
6	P6	I	48.9
7	P7	II	142.0
8	P8R	II	119.5
9	P8L	II	138.1
10	P9	II	145.6
11	P10	II	180.0
12	P11	II	113.8
13	P12	II	167.2
14	P13	III	113.8
15	P14	III	186.6
16	P15	III	112.9
17	P16	III	141.9
18	P17	III	125.8
19	P18R	IV	394.2
20	P18L	IV	448.3
21	P19	IV	392.5
22	P20	IV	437.5
23	P21R	IV	342.9
24	P21L	IV	356.7
25	P22	IV	66.5
26	P23	IV	76.4
27	P24R	IV	177.6
28	P24L	IV	156.2
29	P25R	IV	149.2
30	P25L	IV	159.6
31	P26	IV	49.7
32	P27R	IV	79.5
33	P27L	IV	101.4
34	P28	IV	81.1

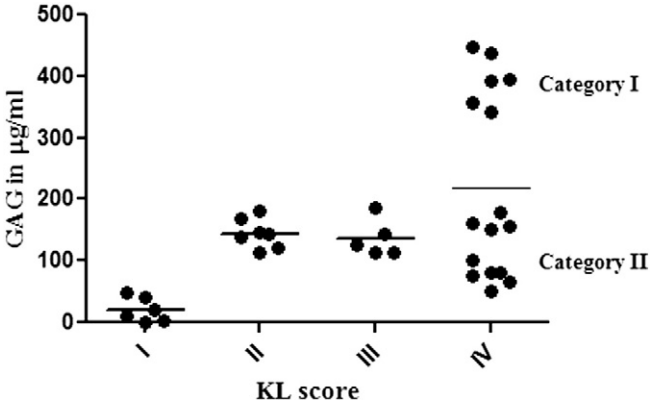

Fig. 1. A graphical presentation of GAG estimation in enrolled OA patients.

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