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Articular cartilage metabolism in patients with Kashin–Beck Disease: an endemic osteoarthropathy in China

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Summary

Objective: The objective of this study was to investigate CD44 and proteoglycan metabolism in patients suffering from Kashin–Beck Disease (KBD), an endemic osteoarthropathy that affects 2.5 million of 30 million people living in the KBD regions of China.

Methods: Immunohistochemical analyses of cluster of differentiation-44 (CD44), BC-13 and 3-B-3(–) expression were performed in cartilage sections harvested from KBD and normal patients. In addition, the serum levels of soluble CD44 (sCD44), interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and matrix metalloproteinase-1 were determined using a sandwich enzyme linked immunosorbent assay.

Results: Hematoxylin & eosin and toluidine blue staining indicated that there was cell necrosis and proteoglycan loss in cartilage from both KBD children and adult cartilage. Strong immunohistochemical staining for CD44, BC-13 and 3-B-3(–) occurred in the majority of adult KBD patients and most KBD children. Furthermore, statistically significant elevated levels of sCD44, IL-1β and TNF-α were found in the sera of both adult and child KBD patients when compared to the levels of normal adult and child controls. Interestingly, IL-1β and TNF-α serum levels were all high in normal children from KBD regions when compared to normal children from non-KBD regions suggesting that unidentified factors (e.g., a genetic predisposition) may protect some people from KBD pathology.

Conclusion: Our results demonstrate that altered CD44, IL-1β and TNF-α metabolism occurs in the pathogenesis of KBD and there is an increased aggrecanase-generated proteoglycan loss from KBD adult and child cartilage. These primary metabolic changes are likely to be significant contributing factor causing pathological joint formation and instability that leads to secondary osteoarthritis in KBD patients.

Key words: Kashin–Beck Disease, CD44, Aggrecanase, Aggrecan metabolism, IL-1β, TNF-α, Osteoarthritis, Proteoglycan, Osteoarthropathy.

Introduction

Kashin–Beck Disease (KBD) is an endemic, chronic, and degenerative osteoarthropathy, which is manifests primarily in agricultural regions of Asia especially in the central regions of the PR China. KBD often occurs in children and is age-related. The symptoms include joint pain, morning stiffness in the joints, disturbances of flexion and extension in the elbows, enlarged inter-phalangeal joints and limited motion in the middle-sized and large joints of the body (Fig. 1). Pathologically, the epiphyseal plate of cartilage from young and adolescent patients shows necrosis in the hypertrophic layer near the adjacent subchondral bone thus explaining why these young KBD patients often have severe joints' deformities during development[1]. At present the etiology of KBD is unclear. One of the most popular hypotheses is that KBD is caused by fungal mycotoxins on stored food, especially T-2 toxin[2]. Other etiologies occur including selenium deficiency in soil and water in the KBD areas; nutrition deficiency and virus infections. Nonetheless, all of these hypotheses still lack adequate experimental evidence to support their proposal.

Osteoarthritis (OA) is a degenerative joint disorder that predominantly occurs in older people worldwide[3]. It has been suggested several years ago that a high prevalence of primary OA occurs in the KBD areas[4]. Thus, one might hypothesize that, in individuals moving into an endemic KBD area during adult life after growth plate closure, their articular cartilage would be susceptible to damage from the KBD causing agents resulting in the onset of a generalized secondary

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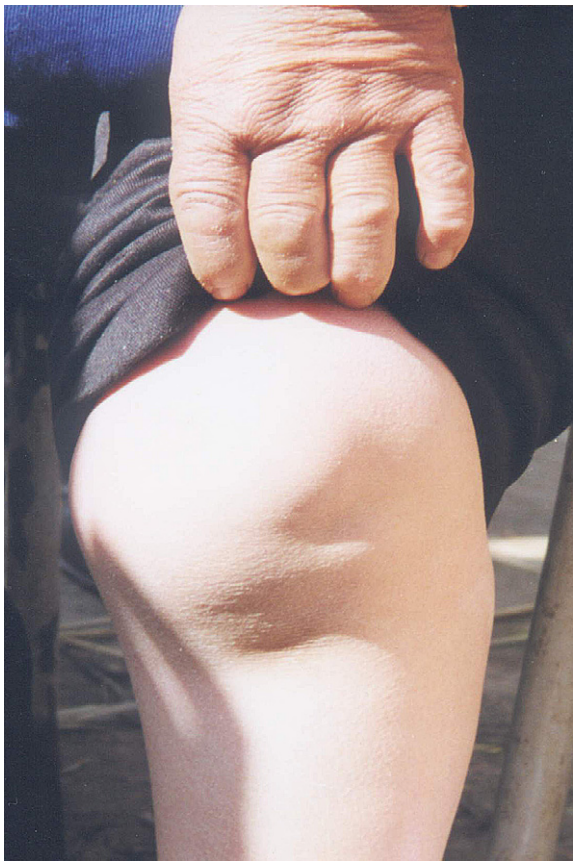

Fig. 1. Enlarged hand and knee joints of KBD patient. The patient was female, 56 years old, and was diagnosed as KBD stage III by X-ray.

OA. Although there have been very few studies investigating this hypothesis, there is a possible relationship between KBD and OA in their pathogenic pathways in spite of their different etiologies.

One of the central pathophysiological features contributing to cartilage erosion during OA is the catabolism and loss of the aggregating cartilage proteoglycan aggrecan[5]. Aggrecan exists as large aggregates in cartilage matrix *via* the interaction of its G1 domain with hyaluronan (HA) and link protein[6–8]. It has been confirmed that the accelerated loss of the aggrecan, and the consequent loss of glycosaminoglycan-bearing aggrecan fragments from articular cartilage, is an early event in the destruction of the articular cartilage seen in the pathogenesis of OA and rheumatoid arthritis (RA)[9]. The loss of aggrecan results in a decrease in functional and structural integrity of the cartilage matrix, which consequently makes the tissue lose its capacity to resist compression under load, eventually leading to irreversible mechanical and further enzymatic destruction of the cartilage[10]. Many proteolytic enzymes are involved in the process including a disintegrin-like and metalloprotease with thrombospondin motifs-4 and -5 (ADAMTS-4 and -5) and matrix metalloproteinases (MMPs). Several studies have identified the aggrecanases as the major proteases responsible for aggrecan degradation and loss from the cartilage at the early stage of degenerative joint disease[5,9,10]. The major proteolytic cleavage site responsible for cartilage pathology occurs in the interglobular domain (IGD) between Glu^{373} and ^{374}Ala[5,10]. Monoclonal and polyclonal antibodies are available to detect the neoepitopes generated at the aggrecanases and MMP cleavage sites in aggrecan[5,10].

Cluster of differentiation-44 (CD44) is a membrane glycoprotein and the primary HA receptor on chondrocytes in articular cartilage. It contains a hydrophobic transmembrane domain followed by a 70-amino acid intracellular domain[11]. The intracellular domain of CD44 contains motifs with the potential for transducing signals and controlling the spatial organization of the receptor in the plasma membrane, which is very important for cell–matrix interactions and proteoglycan retention around the chondrocyte. There is an accumulating evidence suggesting that perturbation of CD44 metabolism occurs during OA pathogenesis, although it is not clear whether this abnormal metabolism is a primary factor or just a consequence of the overall altered cartilage metabolism[12–14].

KBD is a unique endemic arthropathy that appears to have a different etiology when compared with OA, but it does have similar pathological outcome involving cartilage matrix degradation, leading to joint destruction. To date, there have been no studies investigating the metabolism of aggrecan and CD44 in KBD patient articular cartilage. In this study, we found that there was significant aggrecanase-mediated proteoglycan degradation in both adult and child KBD patients and altered CD44 metabolism was also involved in KBD pathogenesis.

Patients and methods

MATERIAL SOURCES

Child KBD cartilage samples were obtained from finger joints from four KBD patients aged from 3 through 7 years old with documented X-ray diagnosis of KBD. These children had died from accidents or other diseases such as bacillary dysentery. The adult cartilage samples were obtained from 16 KBD patients undergoing joint replacement surgery and were aged from 35 through 63 years old. The cartilage samples from the normal children and adults were obtained from patients who had died from clinical problems not involving joint pathology. Ethical approval for acquisition of these patient samples was approved by The Human and Ethical Committee for Medical Research at Xi'an Jiaotong University, School of Medicine (Dr Yong Liu, Director); documents have been provided to the Osteoarthritis Research Society International (OARSI). Ethics Committee with this paper submission. Both pathological (KBD) and normal cartilage samples were obtained within 2–4 h of death and fixed in 4% paraformaldehyde. KBD patient serum samples came from a separate KBD epidemiological survey where participants were subjected to X-ray diagnosis and blood collection. There were 18 KBD children (4–12 years old) samples, 18 normal children from a KBD area (4–12 years old) samples and 20 KBD adult samples (28–55 years old) from KBD areas of Shaanxi Province, PR China. Normal serum samples were obtained from people undergoing routine health examination in a non-KBD region. All of the patients and normal samples were obtained after getting the patient or guardian's consent as authorized by the Ethics Committee of Ministry of Health, Shaanxi Province.

IMMUNOHISTOCHEMICAL STAINING FOR CD44 AND NEOEPITOPES OF AGGRECAN METABOLISM

Cartilage was dissected from the subchondral bone, paraformaldehyde-fixed and then the paraffin-embedded cartilage samples were cut into 6-μm sections and placed on poly-L-lysine-coated glass slides. Immunohistochemical staining was performed with primary antibodies, biotin-conjugated secondary antibodies followed by detection using the strept–avidin–biotin–peroxidase complex (SABC) method (SABC kits: Boster Co, Wuhan, China). Briefly, after deparaffinization, endogenous peroxidase was blocked with 3% H_2O_2 for 15 min and the slides were washed with 0.01 M phosphate-buffered saline (PBS). The slides were then predigested using prewarmed (37°C) 0.1% trypsin (Maixin-Bio Co, Fuzhou, China) or 10 U/ml chondroitinase ABC (Sigma, USA), followed by rinsing with distilled water. After blocked using 10% normal goat serum, the sections were incubated with monoclonal antibodies recognizing either CD44 (Boster Co, Wuhan, China), BC-13 (Abcam, Cambridge, UK) or 3-B-3 (Seikagaku, Japan) or PBS as negative control for 18 h at 4°C followed by incubation with 1:200 biotinylated goat anti-mouse IgG (Boster Co, Wuhan, China). After incubation with the SABC complex, the sections were stained with 3-amino-9-ethylcarbozole. Counterstaining was performed with hematoxylin.