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Research Article

Disordered glycometabolism involved in pathogenesis of Kashin–Beck disease, an endemic osteoarthritis in China

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ABSTRACT

Kashin–Beck disease (KBD) is a chronic endemic osteoarthritis in China. Previous studies have suggested a role of metabolic dysfunction in causation of this disease. In this investigation, the metabolomics approach and cell experiments were used to discover the metabolic changes and their effects on KBD chondrocytes. Nuclear magnetic resonance (^{1}H NMR) spectroscopy was used to examine serum samples from both the KBD patients and normal controls. The pattern recognition multivariate analysis (OSC–PLS) and quantitative analysis (QMTLS iterator) revealed altered glycometabolism in KBD, with increased glucose and decreased lactate and citrate levels. IPA biological analysis showed the centric location of glucose in the metabolic network. Massive glycogen deposits in chondrocytes and increased uptake of glucose by chondrocytes further confirmed disordered glycometabolism in KBD. An *in vitro* study showed the effects of disordered glycometabolism in chondrocytes. When chondrocytes were treated with high glucose, expression of type II collagen and aggrecan were decreased, while TNF-α expression, the level of cellular reactive oxygen species and cell apoptosis rates all were increased. Therefore, our results demonstrated that disordered glycometabolism in patients with KBD was linked to the damage of chondrocytes. This may provide a new basis for understanding the pathogenesis of KBD.

Abbreviations: BCAAs, branched-chain amino acids; DCFDA, dichlorodihydrofluorescein diacetate; DMEM, Dulbecco's modified Eagle's medium; FCM, flow cytometer; GLUT-1, glucose transporter-1; HA, hyaluronic acid; IPA, ingenuity pathways analysis; KBD, Kashin–Beck disease; NMR, nuclear magnetic resonance; OA, osteoarthritis; OSC–PLS, orthogonal signal correction–partial least squares; PI, propidium iodide; QMTLS, quantum mechanical total-line-shape; ROS, reactive oxygen species; TEM, transmission electron microscope; TNF-α, tumor necrosis factor-α; TSP, sodium salt of 3-trimethylsilylpropionic acid

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Introduction

Kashin–Beck disease (KBD) is a human form of chronic and endemic osteoarthritis (OA) found mainly in China, with a geographical distribution covering an oblique belt running from southeastern Siberia in Russia to southwest China [1,2]. In 2010, there were more than 695,000 KBD patients with 105.84 million residents at risk in 366 endemic counties in China (Chinese Health Statistical Digest 2010. http://www.moh.gov.cn/publicfiles//business/htmlfiles/zwgkzt/ptjty/digest2010/index.html). KBD mostly occurs in children, expressed in deformed joints and growth retardation, and it results in severe arthralgia and impaired mobility [3] (Fig. 1A). In adults, the deformed joints seriously impact the health-related quality of life of patients. At present, the diagnosis of KBD is mainly dependent on clinical symptoms and radiographic signs in the hands (Fig. 1B, X-ray image of right hand of an adult). However, radiography only indirectly measures changes in cartilage and the extent of articular cartilage alteration, namely, joint space narrowing, and it usually poorly correlates with joint function; it lags behind molecular and biochemical changes and is less indicative of the dynamic progress of the disease. Metabolomics has unique advantages in this regard. This approach can monitor biochemical changes over time, and metabolic characteristics are closely identifiable with real biological end-points, providing a coherent interpretation of biological effects.

KBD and OA are both debilitating joint diseases characterized by disruption of articular cartilage, degradation of extracellular matrix, cell apoptosis and necrosis, and joint dysfunction [1]. Although KBD has similar pathological outcomes with OA, it is a unique endemic arthropathy with a different etiology. The etiology of KBD is linked to environmental factors, while OA is highly related with aging [4,5]. Pathological findings in KBD clearly reveal a focal chondronecrosis in the deep zone and extracellular matrix degradation in both growth plate cartilage and articular cartilage [6]. The more advanced symptoms are secondary deforming osteoarthrosis and impaired skeletal development. Mounting evidence has confirmed that osteoarthropathia, including the special type known as KBD, is associated with metabolic disorders [7,8]. According to previous studies, a variety of pathophysiological events seen in KBD are related to metabolic changes. For example, the functions of 13/79 (16.5%) of genes that were differentially expressed between KBD and normal chondrocytes were related to metabolism [9], and the functions of 10/75 (13.3%) genes that were differentially expressed between KBD and OA chondrocytes were also associated with metabolism [10]. Disturbances in selenium metabolism, lipid peroxidation and cellular metabolic functions, together with cell membrane damage, metabolism dysregulation and changes in cartilage collagen have all been suggested to play important roles in the pathophysiological progression of KBD [11]. In addition to the above findings, *in vivo* and *in vitro* studies suggest that several cellular and molecular mediators, such as glucose [12], fatty acids [8], growth factors [13], cytokines [14] and free oxygen radicals, may also participate in the chondrocyte damage associated with OA. Osteonecrosis of the femoral head is associated with decreased glucose and lactate concentrations in synovial fluid [15]. The urinary metabolite profile associated with OA could be distinguished from that of normal controls, as well as displaying a strong correlation between the metabolite profile and radiographic OA severity [16].

Based on these published studies, we hypothesized that the pathophysiological changes of KBD would be reflected in the patients' metabolism. In the present study, we aimed to

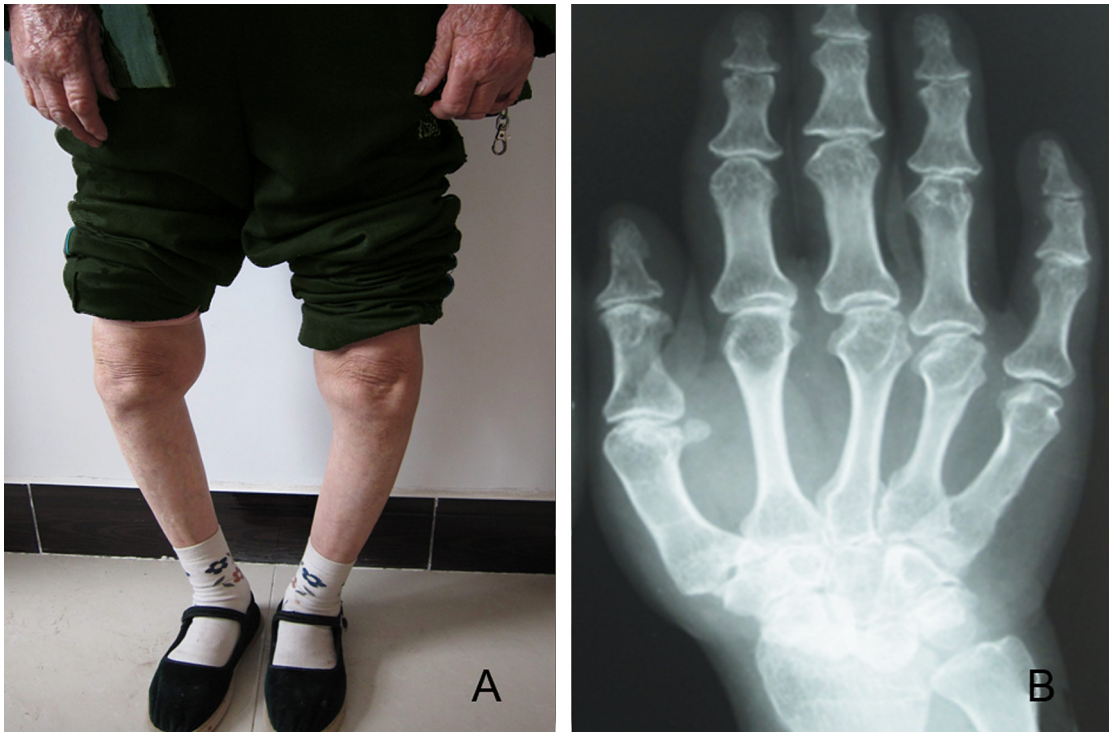

Fig. 1 – Image of KBD patient and radiographic signs in the hands. (A) A KBD patient with deformed knee joints. (B) Radiographs of the right hand of a male adult aged 50 years.