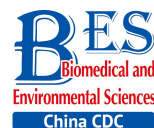


## Letter to the Editor



# Detection of the Urinary Biomarkers PYD, CTX-II, and DPD in Patients with Kashin-Beck Disease in the Qinghai Province of China\*

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Kashin-Beck disease (KBD) is an endemic degenerative osteoarthropathy of uncertain etiology. The aim of our study was to identify changes in C-telopeptide of type II collagen (CTX-II), pyridinoline (PYD), and deoxypyridinoline (DPD) among KBD patients. 54 KBD patients and 78 healthy controls were included in this study. Urinary samples were collected and measured by ELISA. The median quantities of PYD, CTX-II, and DPD of KBD patients were 1107.73 ng/μmol.cre, 695.11 ng/μmol.cre, and 1342.34 pml/μmol.cre, while the median quantities of healthy controls were 805.59 ng/μmol.cre, 546.47 ng/μmol.cre, and 718.15 pml/μmol.cre, respectively. The differences between KBD patients and healthy controls were statistically significant ( $Z = 4.405, 3.653, \text{ and } 3.724; P < 0.001$ ). The higher levels of PYD, CTX-II, and DPD detected in KBD patients indicate that they could be used as biomarkers of KBD.

Kashin-Beck disease (KBD) is an endemic degenerative osteoarthropathy characterized by chondrocyte necrosis, apoptosis, cartilage degeneration, and matrix degradation<sup>[1]</sup>. The pathological changes of KBD progress slowly and lead to secondary osteoarthritis. KBD is geographically distributed in a narrow zone in China from the northeast to the southwest. The etiology of KBD remains uncertain. Three major environmental causes have been proposed: selenium deficiency, cereal contamination by mycotoxin-producing fungi, and high levels of organic material in drinking water.<sup>[2-3]</sup>

Usually the development of KBD is monitored by X-ray of the hand, however this method only measures bone erosion and cartilage loss with poor sensitivity. Radiographic changes develop over relatively long periods of time and significant

cartilage degradation must occur before it is seen on a radiograph<sup>[4]</sup>. Magnetic resonance imaging (MRI) is also used to assess quantitative and qualitative cartilage changes<sup>[5]</sup>, however this is an expensive and time-consuming method.

Early diagnosis and treatment of KBD is paramount in preventing joint damage and disability. Biochemical markers are molecules derived from connective tissue fragments, which can be measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to therapeutic interventions<sup>[6]</sup>. Sensitive biomarkers reflecting the turnover and activity of the synovium, cartilage, and bone tissues can be used to monitor disease progression and drug efficacy more reliably and with higher sensitivity in comparison with radiographic changes which often occur slowly<sup>[5]</sup>.

Type II collagen is the most abundant constituent of the cartilage matrix. The C-telopeptide of type II collagen (CTX-II) can be measured in urine providing a sensitive and specific measure of type II collagen breakdown<sup>[7]</sup>. Pyridinoline (PYD) and deoxypyridinoline (DPD) are bone turnover markers that can be detected in urine<sup>[8]</sup>. The aim of our study was to identify changes in the levels of CTX-II, PYD, and DPD in urine in KBD patients. This investigation might provide a better understanding of KBD pathogenesis.

The study was carried out in compliance with the ethical principles outlined in the world medical association Helsinki's declaration. This study was approved by the Ethics Committee of the Qinghai Institute for Endemic Disease Prevention and Control. The consent procedure was approved by the Ethics Committee of the Qinghai Institute for Endemic Disease Prevention and Control.

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The study was carried out in April 2013. Patients were diagnosed with KBD based on the national diagnostic criteria of KBD in China (WS/T207-2010). This includes living in KBD endemic areas in Xinghai and Guide counties along joint changes and specific changes on X-ray. Individuals with rheumatoid arthritis (RA), primary osteoarthritis (OA), skeletal fluorosis, and gouty arthritis were excluded. Normal controls included individuals with normal joints at physical examination and no other bone or joint diseases. Normal controls were also from KBD endemic areas in Xinghai and Guide counties.

Early morning urine samples were obtained from all study participants after clinical and X-ray examinations. Urinary samples were frozen at -80 °C until analysis. Urinary CTX-II, PYD, and DPD were measured by enzyme linked immunosorbent assay (ELISA) provided by Shinghai Shifeng Biological technology Co. Ltd. following the supplier’s protocols. Optical densities were determined using an automatic microplate reader (Rayto Co, China). Intra- and inter-assay CVs were 9% and 15% for all the biomarkers, respectively.

Statistical analysis was performed using the SPSS software, Version 17.0. Ages were expressed as mean ± standard deviation (SD) and compared between groups using Student’s *t*-test. The levels of CTX-II, PYD, and DPD in each group were expressed as median, range, and quartile range and analyzed using the Wilcoxon rank sum test. Results were considered statistically significant if the *P* value was less than 0.05.

A total of 132 participants were included in the study. 54 cases were diagnosed as KBD (21 males and 33 females) and 78 individuals were included as healthy controls (34 males and 44 females). There were no statistically significant differences in age between the two groups (46.54 ± 16.06 years of age of KBD patients and 49.49 ± 14.24 years of age of healthy controls) (*P* > 0.05). There were also no statistically significant differences in age between females and males of each study group (male KBD patients 47.42 ± 13.31 years old and male healthy controls 51.88 ± 12.29 years old, *P* > 0.5; female KBD

patients 45.97 ± 14.69 years old and female healthy controls 47.59 ± 15.04 years old, *P* > 0.05) (Table 1).

The main clinical signs of the KBD patients in this study included finger flexion and enlargement and deformation of the finger joint. The main X-ray signs were osteophyte, narrowing of the joint space, bone deformity, enlargement of the phalanx, and carpal crowding. The urinary median of PYD, CTX-II, and DPD of KBD patients was 1107.73 ng/μmol.cre, 695.11 ng/μmol.cre, and 1342.34 pml/μmol.cre, respectively, while the urinary median of PYD, CTX-II, and DPD of healthy controls was 805.59 ng/μmol.cre, 546.47 ng/μmol.cre, and 718.15 pml/μmol.cre, respectively. There were statistically significant differences in three biomarkers between KBD patients and healthy controls (*Z* = 4.405, 3.653, and 3.724, *P* < 0.005) (Table 2).

China has the highest number of KBD patients in the world. 38,071,000 people are under threat of developing KBD in China. 16,826 of a total 644,994 KBD affected individuals are children under the age of 13<sup>[4]</sup>. Knee pain and functional limitations are the main factors affecting the lives and abilities of KBD patients. The degenerative changes observed in the cartilage of KBD patients include surface fibrillation, chondrocyte necrosis and apoptosis, loss of proteoglycans and collagen II degradation from the matrix<sup>[1]</sup>. Among these, type II collagen levels in chondrocytes from KBD patients are significantly lower than those from healthy controls<sup>[10]</sup>. KBD has a similar pathological outcome regarding cartilage matrix degradation with osteoarthritis (OA)<sup>[9]</sup>. Thus, research on OA is also useful for research on KBD. Due to the high prevalence of OA and its uncertain pathogenesis, the identification of biomarkers is important for monitoring, early diagnosis, and assessment of therapeutic effects.

CTX-II has been regarded as a potential prognostic indicator of OA progression and articular cartilage degradation<sup>[7]</sup>. The urinary level of CTX-II is strongly correlated with overall cartilage degradation of the hip, hand, facet, and knee joints<sup>[5]</sup>. The serum levels of CTX-II were higher in KBD patients than in the control group<sup>[10]</sup>. Type II collagen

**Table 1.** Age Comparison between KBD Patients and Healthy Controls (mean ± SD)

Gender	Healthy Control		KBD Patients		<i>T</i>	<i>P</i>
	Number	Age	Number	Age		
Male	34	51.88 ± 12.29	21	47.42 ± 13.31	1.265	<i>P</i> > 0.05
Female	44	47.59 ± 15.04	33	45.97 ± 14.69	0.473	<i>P</i> > 0.05
Total	78	49.49 ± 14.24	54	46.54 ± 15.06	1.163	<i>P</i> > 0.05

**Table 2.** The Urinary Levels of PYD, CTX-II, and DPD between KBD Patients and Healthy Controls

Biomarkers	Gender	Patients				Healthy Control				Z	P
		Median	Range	25%Q	75%Q	Median	Range	25%Q	75%Q		
PYD (ng/μmol.cre)	Female	1106.39	438.82-1918.70	757.1	1340.35	702.53	272.55-1597.75	468.17	1086.32	3.858	P < 0.001
	Male	1151.5	593.14-3042.05	875.4	1167.35	756.51	295.68-1522.33	529.14	987.38	3.567	P < 0.001
	Total	1107.73	438.82-3042.05	843.09	1394.59	805.59	272.55-1597.75	533.65	1115.62	4.405	P < 0.001
CTX-II (ng/μmol.cre)	Female	677.01	335.95-1576.51	524.79	837.89	534.46	144.36-1239.97	266.33	639.26	3.432	P < 0.001
	Male	704.89	431.39-2159.32	517.45	1199.44	452.27	165.69-984.21	368.12	657.36	3.618	P < 0.001
	Total	695.11	335.95-2159.32	520.35	920.85	546.47	144.36-1239.97	363.7	731.47	3.653	P < 0.001
DPD (pml/μmol.cre)	Female	1364.13	430.06-3713.06	918.03	1838.44	602.68	86.42-1433.27	358.94	815.79	4.563	P < 0.001
	Male	1438.81	203.24-3166.29	684.97	2312.85	743.04	88.35-1646.35	313.13	1151.32	2.017	P < 0.050
	Total	1342.34	203.24-3713.06	696.99	2208.67	718.15	86.42-1646.35	375.22	1025.72	3.724	P < 0.001

could effectively reduce the serum level of CTX-II and delay the process of articular cartilage damage induced by T-2 toxin. In this study, the urinary levels of CTX-II in KBD patients were higher than in the control group ( $P < 0.001$ ), indicating that articular cartilage degradation is involved in the pathogenesis of KBD. This finding is consistent with a previous study<sup>[6]</sup>.

PYD and DPD derive from the breakdown of collagen. PYD and DPD have been validated as useful markers for bone resorption and their levels are significantly higher in OA patients<sup>[8]</sup>. Due to their specific localization in bones and renal excretion, the measurement of urinary levels of PYD and DPD could provide insight into bone metabolism and help us better understand pathological changes in bone and cartilage<sup>[8]</sup>. In this study, the urinary levels of PYD in KBD patients were also higher than in healthy controls ( $P < 0.001$ ). This indicates that collagen breakdown of the articular cartilage occurs in KBD patients, which are consistent with a previous study.

In this study, we found that there were biological changes in the urine of KBD patients. Although more biomarkers can be identified in serum, serum collection is invasive. Because of language and culture barriers in Qinghai, the local population does not accept invasive methods of examination. Thus, prevention and control of KBD is lagging behind. Detection of biomarkers in urine samples provides a non-invasive method to monitor KBD. However, the results presented here should be taken with caution since the sample size was small and only a few biomarkers were measured.

In summary, we found that there were changes in urinary biomarkers in KBD patients. The total urinary levels of PYD, CTX-II, and DPD in KBD patients were higher than healthy controls. These biomarkers could reflect the changes of collagen breakdown of KBD. Therefore, biomarkers in urine will help to better understand KBD pathogenesis in the future.

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All other Authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal. No interest conflicts existing in this submission.

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All patients and controls signed an informed consent form. This consent procedure has been approved by the Ethics Committee of the Qinghai Institute for Endemic Disease Prevention and Control.

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