Letter to the Editor

A Primary Investigation on Serum CTX-II Changes in Patients Infected with Brucellosis in Qinghai Plateau, China*

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Brucellosis is one of the most widespread zoonotic diseases, with the most frequent complication being osteoarticular changes. The aim of this study was to assess the changes of C-terminal telopeptide of type II collagen (CTX-II) in patients infected with brucellosis. A total of 84 brucellosis patients and 43 volunteers were selected and divided into brucellosis *vs.* control groups. Serum samples were subjected to serological tests for brucellosis, and CTX-II levels in all samples were measured simultaneously with ELISA. The results showed that serum CTX-II levels in human brucellosis were higher than those of healthy controls, without a statistically significant difference, but serum CTX-II levels in male patients were significantly higher than those of female patients (P<0.05). This finding could indicate the biological changes in the cartilage and bone in human brucellosis.

Brucellosis, one of the most widespread zoonotic diseases worldwide, not only affects both wild and domestic animals but also poses severe threats to human health. Human brucellosis is transmitted through contact with infected animals or consumption of contaminated foods. The clinical manifestations vary from joint, muscle, and back pain to flu-like symptoms and even more serious conditions implicating different organ systems[1]. The diagnostic methods of human brucellosis include the isolation of *Brucella* spp., serological tests for the detection of anti-*Brucella* spp. antibodies, and the detection of *Brucella* spp. DNA[2]. The serological tests include the serum agglutination test (SAT), Rose Bengal plate agglutination test (RBPT), complement fixation test (CFT), and the enzyme-linked immune sorbent assay (ELISA) for IgG and IgM.

Brucellosis is a global disease. In Europe, this zoonosis remains endemic in several Mediterranean countries and is a severe public health problem. Brucellosis has been prevalent for a long time in Mainland China, and human brucellosis was distributed in 25 of 32 provinces (or autonomous regions) of Mainland China. Consequently, this disease was under the prevention and control programs. With the rapid development of China's animal husbandry in recent years, the incidence of human brucellosis had increased sharply, and >164,752 people were reported to be infected with brucellosis from 2004 to 2010[3]. The Chinese Center for Disease Control and Prevention (China CDC) had reported 39,515 new cases of human brucellosis in 2012, with the number of new cases increasing by 10% each year. At present, human brucellosis is also considered as an important public health problem in Mainland China. The Qinghai province is the active region of human brucellosis in Mainland China, since the recurrence and prevalence of human brucellosis in 2006, and the prevalence rate of human brucellosis in Qinghai province was 1.67% in 2012.

Although several organs and organ systems may be involved, osteoarticular changes are observed as the most frequent complication of brucellosis. The spectrum of musculoskeletal manifestations of brucellosis includes sacroiliitis, spondylitis, arthritis, osteomyelitis, and bursitis[4]. Therefore, human brucellosis presents various diagnostic difficulties, because it mimics several other diseases. Radiological examinations were one of the important instruments for determining osteoarticular involvement in brucellosis[5], but the long latent period between the occurrence of symptoms and the appearance of radiological changes may delay the early diagnosis of brucellosis[6]. Biomarkers are molecules or fragments that are released into biological fluids during the process of tissue turnover.

doi: 10.3967/bes2016.029

*This study was supported by the National Natural Scientific Foundation of China (30972556).

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In osteoarthritis (OA) and rheumatoid arthritis (RA), biomarkers were regarded as the index of joint damage for evaluation of the effect of treatment modalities on OA and RA[7]. The C-terminal telopeptide of type II collagen (CTX-II) is a biomarker of type II collagen degradation[8]. CTX-II levels were elevated in both RA and OA patients as compared with normal individuals[9], and CTX-II levels were correlated with the extent of joint destruction and can help predict progression[7]. Human brucellosis could cause musculoskeletal changes; however, only few studies have prospectively related biomarker measures of cartilage or bone to human brucellosis. The aim of this study was to identify the correlation between CTX-II levels and human brucellosis. Such data should provide a better understanding of the osteoarticular changes inhuman brucellosis.

This study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki of the World Medical Association and was approved by the Ethics Committee of the Qinghai Institute for Endemic Disease Prevention and Control. All patients and controls signed an informed consent form, which was approved by the Ethics Committee of the Qinghai Institute for Endemic Disease Prevention and Control. The study included a total of 84 brucellosis patients (47 males and 37 females), with an age range from18 to 72 years (mean±SD: 39.40±10.92 years). The SAT, RBPT, and CFT were performed following the methods described by the diagnostic criteria for brucellosis (WS269-2007). ELISA was conducted for IgG and IgM assays. The inclusion criterion was the identification of the antibody titer to *Brucella* with SAT (the identification of<1/100 antibody titer to *Brucella*), in association with a compatible clinical finding. Subjects diagnosed with rheumatic fever, typhoid fever, paratyphoid fever, RA, and tuberculosis were excluded from the study. The control group included 43 volunteers (23 males and 20 females), whose ages ranged from 22 to 72 years (mean±SD: 43.96±10.46 years). Subjects in the control group were negative for SAT. The exclusion criterion for the healthy control cases was any abnormalities of osteoarticular involvement. Venous blood samples were collected from all study subjects and were centrifuged at 3000 rpm for 15 min. The serum samples were separated and stored at -80 °C until assayed. CTX-II levels in all the serum samples were measured simultaneously usinga commercial ELISA kit, according to the kit procedure (Shifeng Co. Ltd, Shanghai, China). The results were expressed as ng/mmoL.

The means and standard deviations were calculated for each parameter, and data were analyzed for normal distribution. The means of variables showing normal distribution were compared by the parametric unpaired *t*-test. A *P* value <0.05 was considered to bestatistically significant. Data were analyzed using SPSS 17.0 software (SPSS, Chicago.IL, USA).

Comparison of the general characteristics of brucellosis patients and healthy controls showed no significant differences in age and gender (Table 1). The RBPT is commonly used as a screening test for human brucellosis cases, and hence the brucellosis cases in the present study were confirmed by SAT. All the human brucellosis cases were positive for RBPT and SAT (Table 2). Meanwhile, 38.09% (32/84) of human brucellosis cases tested positive with CFT. In contrast, 94.05% (79/84) and 16.67% (14/84) of human brucellosis cases were positive for IgG and IgM, respectively, with a statistically significant difference between the two antibodies (χ^2=101.763, *P*<0.001), suggesting that most of the cases have been infected with *Brucella melitensis* for a long time.Human brucellosis is a chronic disease with multi-systemic involvements, which present with a variety of symptoms. *B. melitensis*, *B. abortus*, *B. suis*,

Table 1. General Characteristics of Brucellosis Patients and Healthy Controls

Characteristics	Patient	Control	*P* Value
Gender (no.)			
Male	47	23	
Female	37	20	
Total	84	43	
Age (mean±SD)			
Male	39.70±10.86	43.96±10.46	*P*>0.05
Female	39.02±11.13	41.70±7.77	*P*>0.05
Total	39.40±10.92	42.91±9.27	*P*>0.05

and *B. canis* are responsible for causing human brucellosis[2]. At present, the topic of research interest is on how to improve the diagnosis level of human brucellosis. Traditional serological diagnostic methods of human brucellosis identify only the antibody titer of the patients who are infected with *Brucella*. In the present study, we found that these methods could confirm the 84 patientsas human brucellosis cases.

However, brucellosis patients have various complications, with osteoarticular changes being adominant complication[4]. Osteoarticular manifestations of human brucellosis include arthritis, bursitis, sacroiliitis, spondylitis, and osteomyelitis[6]. Osteoarticular changes can occur in 30%-85% of patients diagnosed with brucellosis[1]. Radiological imaging is used for diagnosing osteoarticular complications of human brucellosis; however, by the time the radiological changes of osteoarticular manifestations are identified, the osteoarticular lesions would have become irreversible, thus indicating the lack of sensitivity of radiological imaging in the early stages[10]. A long diagnostic delay could be a reason for multiple osteoarticular involvement. Therefore, it is necessary to identify the molecular and biological changes in the bone or cartilage in patients infected with brucellosis to make a correct diagnosis in the early stages.

Over the last several decades, only a few studies have analyzed the molecular and biological characteristics of osteoarticular changes among human brucellosis cases. CTX-II shows high sensitivity and specificity for OA and RA[7]. In this study, serum CTX-II levels in human brucellosis cases were higher than those of healthy controls, although without a statistically significant difference (Table 3). CTX-II levels were also higher in both male and female patients than those of healthy controls, again without a statistically significant difference. Intake of drugs in some patients has been considered as a primary reason for decreased serum CTX-II levels in human brucellosis cases. However, to our surprise, we observed in the present study that serum CTX-II levels were significantly higher in male patients than in female patients (t=2.528, P<0.05), whereas serum CTX-II levels in male controls were not higher than those of female controls (t=2.041, P>0.05). Such a finding necessitates further research.

Because this is the first report about the study of serum CTX-II levels in human brucellosis cases, there were a few limitations. First, insufficiency in the osteoarticular data would not show the correlation between serum CTX-II levels and human brucellosis; Second, because of the lack of basic data of patients infected with brucellosis, such as the division of acute, subacute, or chronic and the condition of drug treatment, the changes in serum CTX-II levels could not be explained reasonably. Third, other biomarkers of osteoarticular changes were excluded in this study; thus, the biological characteristics of osteoarticular changes in human brucellosis could not be assessed. However, this study provides a new field for further investigation on human brucellosis.

Although we still could not understand the authentic relationship between CTX-II and human brucellosis, the present study results could not be compared with other published articles. Nevertheless, it provides a new research area for rapidly identifying the osteoarticular manifestations in human brucellosis.

We wish to express our gratitude to WANG Hu and WANG Zu Yun for their assistance with experiments.

Table 2. Results of Traditional Serological Diagnostic Methods of Human Brucellosis Cases and Healthy Controls (+)

Group	RBPT(+)	SAT(+)	CFT(+)	IgG(+)	IgM(+)
Patient	84	84	32	79	14
Control	0	0	0	0	0

Table 3. Results of Serum CTX-II Levels in Human Brucellosis Cases and Healthy Controls

Gender	Patient (serum CTX-II ng/mmoL)	Control (serum CTX-II ng/mmoL)	*P* Value
Male	228.81±275.09	168.02±71.70	P>0.05
Female	119.78±99.09	108.20±46.57	P>0.05
Total	180.78±221.73	140.20±62.34	P>0.05

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Received: November 26, 2015;
Accepted: March 2, 2016

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