

Clinical features and outcomes of brucellosis complicated with Epstein-Barr virus infection

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Research article

Keywords: Brucellosis, EB virus, Clinical analysis, Co-infection, Treatment outcome

Posted Date: December 10th, 2019

DOI: <https://doi.org/10.21203/rs.2.18480/v1>

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Abstract

Objective To analyze the clinical, laboratory characteristics and prognosis of brucellosis patients with co-existing Epstein-Barr virus (EBV) infection at a major hospital in Shandong, a brucellosis epidemic region of China.

Methods A total of 576 inpatients diagnosed with brucellosis at Yidu Central Hospital, between July 2013 and July 2018, were selected and tested for EBV DNA. 22 patients were found to be positive for co-infection with EBV. The clinical data of these 22 patients (observation group) and 100 patients (control group) with only brucellosis were retrospectively compared.

Results The observation group (the group with the EBV co-infection) had more severe clinical manifestations in the form of fever, headache, and hepatosplenomegaly. Further, the observation group also had a significantly higher number of patients with elevated alanine transaminase (ALT) and aspartate-aminotransferase (AST), reduced WBC, and elevated PLT count. The incidence of abnormal levels of cardiac enzyme was also significantly higher in the observation group, as was the recovery time and average hospitalization period.

Conclusions It is important to consider EBV infection and other potentially latent viral infections in patients with brucellosis, as these infections can further complicate the disease course. Further, patients diagnosed with a co-infection should be administered combined antibacterial and antiviral treatment and kept under observation and followed up for a longer period.

1. Introduction

Brucellosis, which is also known as Malta fever, and Mediterranean fever, is an neglected zoonotic disease¹. Human brucellosis is more than 500,000 new cases in the world annually^{1,2}. Brucellosis remains an important zoonoses, which is officially classified as B infectious disease in China. Human brucellosis is endemic in several provinces, including Inner Mongolia, Shanxi, Heilongjiang, Hebei and Xinjiang³. Further, there is evidence that the disease has spread from endemic to non-endemic areas, and has taken on the changing epidemiology. The incidence of brucellosis has been increasing annually since 2001, partly due to the well-developed animal (small ruminants and cattle) husbandry production in Shandong Province^{4,5}.

Brucellosis, which commonly occurs in sheep, cattle, and pigs, is transmitted to humans through many routes, including the digestive and respiratory tract, but it is mainly spread through direct contact with the skin and mucous membrane⁶. It is a complex condition that has diverse clinical manifestations, an atypical onset, and its treatment period is also typically prolonged. Some of the common symptoms are irregular fever, fatigue, excessive sweating, anorexia, chills, muscle pain, joint pain, hepatosplenomegaly, and poor appetite⁷. Early diagnosis is difficult, and misdiagnosis or missed diagnosis is common in non-endemic areas¹. If co-infection with other viruses occurs at the same time, the disease gets further

complicated and is likely to increase in severity. This makes diagnosis and treatment more difficult. A likely agent of co-infection is Epstein-Barr virus (EBV), which is a common γ herpesvirus subfamily that has a high infection rate in population^{8,9}. Often, this virus remains in a latent state and gets activated in a state of immune dysfunction. Further, the immune mechanism of EBV-latent-activated infection is very complicated^{10,11}.

It is worthy to note that brucellosis is also re-emerging in Shandong Province^{12,13}. However, studies report the detail clinical characteristics of human brucellosis from Shandong are lacking. Especially, previous information about brucellosis co-infection with EBV was unclear. In this study, cases of brucellosis with concomitant EBV infection were retrospectively analyzed. The clinical and laboratory features, as well as the treatment and prognosis of the co-infection were discussed for the first time..

2. Patients And Methods

2.1. Hospital and participants

This retrospective study was conducted at the Infectious Diseases Department of Weifang Yidu Central Hospital(WYCH), Shandong. A total of 576 cases of brucellosis were collected between July 2010 and July 2018, and the 22 cases were positive for EB virus.

2.2. Diagnosis

2.2.1 Diagnosis of brucellosis: All the 576 patients were positive with the Rose Bengal Plate Agglutination Test(RBPT), with a titer of 1:100 or above measured in Serum Agglutination Test (SAT). Test reagents were provided and undertaken by the Qingzhou Center for Disease Control and Prevention. Based on the patients' epidemiological history, clinical manifestations, laboratory examination, all the cases met the diagnostic criteria for brucellosis (WS269-2019) and were confirmed as cases of brucellosis.

2.2.2 Diagnosis of EBV infection: Based on the guidelines for diagnosis and treatment of major non-neoplastic EBV infection-related diseases in children¹⁴, patient serum samples were assessed for IgM by ELISA and EBV DNA by PCR analysis. Patients who were positive for other hepadnaviruses (such as hepatitis A, B, and C), cytomegalovirus, were excluded. HIV was not detected in this study.

2.3 Methods

The 22 cases of brucellosis with concomitant EBV infection were analyzed using recorded hospital data, including epidemiology, clinical manifestations and complications. The laboratory examinations and clinical data included blood routine, biochemistry, urine routine, treatment plan, and prognosis. Patients with brucellosis alone (n = 100) were randomly selected as the control group. The age and sex distribution of the two groups was comparable, and the differences in clinical features were compared. The blood routine exam was conducted with the SYSMEX-XE-2100D automatic blood cell analyzer. The Roche COBAS-C-701 automatic biochemical analyzer was used to detect liver and kidney function. The

AMPLLY9800 fully automatic PCR analyzer was used to determine the EBV DNA load by Taqman Real-time PCR. The EBV DNA load of $>1.0 \times 10^2$ IU/ml was considered to indicate EBV infection. Anti-IgM was also detected by ELISA.

2.4 Treatment

For brucellosis, doxycycline was administered two times a day at a dose of 100 mg each time, and rifampicin was administered once a day at a dose of 600 mg according to the guidelines of the Ministry of Health. Based on the temperature and complications of the patient, an alternate regimen recommended by the guidelines is a combination of levofloxacin (0.5 mg once a day), cotrimoxazole (two times a day, 1.0 mg each time), and amikacin (two times a day, 0.4 mg each time). The total duration of treatment was 18 weeks. Patients with concomitant EBV infection were first administered intravenously with ganciclovir (two times a day, at a dose of 5 mg/kg each time). After discharge, the treatment was changed to aciclovir orally, with a total course of 21 days.

2.5 Statistical analysis

All statistical analyses were performed using the SPSS software (version 22.0; SPSS, Chicago, IL, USA). The distribution of variables in the groups was compared with the Chi-squared test or Fisher's exact test for categorical variables. P-values <0.05 were considered significant.

2.6 Ethics issues

The ethics committee of WYCH approved the study. Written informed consent has been obtained from all patients in accordance with the Declaration of Helsinki.

3. Results

3.1 General information

The 22 cases of concomitant infection were considered as the observation group, and this group comprised 10 males and 12 females and had a median age of 45 years (age range, 22–68 years). The control group comprised 100 cases of brucellosis alone, and the age and gender distribution of this group were comparable with that of the observation group. The 22 cases were positive for EBV DNA. The EBV DNA load was between 3.0×10^3 IU/ml– 7.0×10^5 IU/ml, with an average of 4.6×10^4 IU/ml. Anti-IgM antibody was positive in 16 of 22 cases. *Brucella melitensis* was positive in 10 cases from blood.

3.2 Clinical manifestations

As shown in Table 1, the incidence of headache, hepatosplenomegaly and lymphnode enlargement was significantly higher in the observation group.

Table 1
Clinical manifestations in the study cohort (N = 122)

Clinical manifestation	Control group (n = 100)		Observation group (n = 22)	
	Number of cases	%	Number of cases	%
Fever	93	93	22	100
Fatigue	95	95	20	90.9
Sweating	85	85	17	77.2
Anorexia	80	80	19	86.4
Muscle ache	72	72	22	100
Lumbago	38	38	7	31.8
Arthralgia	15	15	6	27.3
Hepatosplenomegaly	8	8	14	63.6 [□]
Lymphnode enlargement	7	7	5	22.7 [□]
Headache	5	5	16	72.7 [□]
*P < 0.05				

3.3 Laboratory data

As shown in Table 2, a significantly higher number of patients in the observation group exhibited a reduction in platelet count and elevation in lactate dehydrogenase levels. However, a significantly lower number of patients in the observation group had elevated lymphocyte/monocytes proportions, elevated AST and ALT levels, and reduced WBC counts.

Table 2
Laboratory findings in the study cohort (N = 122)

Laboratory data	Control group (n = 100)		Observation group (n = 22)	
	Number of cases	%	Number of cases	%
Elevated in ESR	92	92	20	90.9
Elevated in the proportion of monocytes and/or lymphocytes	42	42	16	72.7 [□]
Elevated in CRP	54	54	13	59.1
Elevated in ALT	26	26	17	77.3 [□]
Elevated in PCT	12	12	4	18.2
Reduction in WBC count	15	15	12	54.5 [□]
Reduction in PLT count	7	7	15	68.1 [□]
Elevated in AST	23	23	13	59.1 [□]
Proteinuria	5	5	4	18.2
* P < 0.05				

3.4 Complications

As shown in Table 3, a significantly higher number of patients in the observation group had abnormal hepatic enzymes and cardiac enzyme.

Table 3
Prevalence of complications in the study cohort (N = 122)

Complication	Control group (n = 100)		Observation group (n = 22)	
	Number of cases	%	Number of cases	%
Abnormal hepatic enzymes	26	26	17	77.3 [□]
Vertebrae	23	23	3	13.6
Arthritis	13	13	2	9
Abnormal Cardiac enzyme	12	12	15	68.2 [□]
*P < 0.05				

3.5 Follow-up and outcome

Satisfactory results were obtained in both groups. The therapeutically failure and relapses were not reported in our study after 18-weeks treatment. However, as evident in the Table 4 below, the recovery period and hospital stay were significantly longer in the observation group. The fever in the observation group lasted for a long time, and the abnormal hepatic enzymes and cardiac enzyme, were higher than those in the control group, and the average blood routine recovery and hospital stay were prolonged. EBV DNA load was all less than $1.0 \cdot 10^2$ IU/ml and anti- IgM was all negative after 1 months follow-up.

Table 4
Treatment outcomes in the study cohort (N = 122)

Outcome indicator	Control group (n = 100)	Observation group (n = 22)
Number of days after which body temperature returned to normal	5.5	8.6
Number of days required for recovery from abnormal hepatic enzymes	10.2	15.5
Number of days required for recovery from abnormal Cardiac enzyme	7.6	12.3
Number of days required for the WBC count to return to normal	8.6	14.7
Number of days required for the PLT count to return to normal	9.5	17.6
Average number of days of hospitalization	11.5	16.6 [□]

4. Discussion

Shandong province is divided into 17 prefectures, 137 counties, and 1,941 townships. In 2016, the annual incidence rates of human brucellosis rose to 4.1 cases per 100,000 population ¹⁵. The top five of human brucellosis cases were Weifang (675), Heze (401), Jining (398), Jinan (383) and Linyi (374 cases). Most patients from Weifang were diagnosed and treated at WYCH. Since 2010, several large outbreaks have occurred. These outbreaks were considered a consequence of the rapid increase livestock husbandry (mainly sheep), rapid development of dairy industry, and non-effective livestock quarantine. In Shandong, comprehensive procedures for controlling the endemic are lacking, and the effective health promotion from the doctors to the high risk population is inadequate¹⁶. These reasons may primarily explain the high incidence of the disease in this region. Although brucellosis complications are common, the co-infection with EBV have never been reported yet. In the present study, we have compared the features and

outcomes of co-infection with brucellosis and EBV and brucellosis infection alone. The patients with concomitant infection were found to have more severe symptoms, such as recurring fever, headache, hepatosplenomegaly, a high degree of abnormal hepatic enzymes. Because of elevated leukopenia and thrombocytopenia incidence rate, co-infection patients were also misdiagnosed as hematopathia¹⁷. Additionally, the recovery period of the patients with concomitant infection was much longer. EBV infection induces the activation of specific T lymphocytes and the monocyte-macrophage system, as a result of which inflammatory mediators (such as IL-1, IL-6, IL-10, IFN γ , and granulocyte-stimulating factors) are released, a series of inflammatory reactions that impair the function of hepatic sinusoids and endothelial cells are triggered, and the transport system of small bile ducts and bilirubin excretion are hindered¹⁸. These events may result in the elevation of transaminase levels and be responsible for the slower recovery of patients with EBV infection. Accordingly, the pathogenesis of brucellosis has also been found to involve the activation of microglia and cytotoxic T lymphocytes^{19,20}.

In this study, the findings indicate that patients with concomitant EBV infection may have latent EBV infection that is activated under the poor immune conditions resulting from brucellosis. A leukocyte and platelet assay could help determine whether concomitant EBV infection is present in patients with brucellosis. Additionally, drugs against *Brucella* would not be ideal in cases of concomitant EBV or other viral infections, as these patients would require a combined antiviral and antibacterial regimen. Patients with co-infection should also be followed up for a longer time to ensure that there is no latent infection. For all these reasons, the possibility of concomitant EBV infection or other viral infections should be seriously considered in patients with brucellosis.

Importantly, it was found that the probability of abnormal hepatic enzymes and cardiac enzyme was significantly elevated in the concomitant infection group. Rifampicin, a first-line drug for brucellosis, could also lead to abnormal hepatic enzymes. It is necessary to avoid rifampicin in the concomitant cohort. Therefore, second-line drugs and hepatoprotective drugs needed to be used at the same time, which led to the prolongation of treatment cycle and the increase of treatment cost.

5. Conclusions

This study describes the clinical, laboratory characteristics and prognosis of brucellosis patients with co-existing Epstein-Barr virus infection in Shandong, China. Given that brucellosis is still endemic in this region, it is important to consider EBV infection and other potentially latent viral infections in patients with brucellosis.

Limitation of the study:

The present study is retrospective in nature. Additionally, since most patients were not followed up regularly, the changes in EBV DNA load in the patients with concomitant infection could not be monitored after treatment. Finally, this study is the small number of samples, as this may have caused a bias.

Nonetheless, the findings provide important clinical information about patients with brucellosis who have concomitant EBV infection.

Declarations

Authors' contributions: Hongyan Zhao and Chaoxia Sun were the principal researchers, conceived the study, designed and collected data, conducted laboratory works and drafted the

manuscript for publication. Xiuying Ni involved in data collection, laboratory works and data analysis. Li Zhao and Xuejun Dong reviewed the initial draft manuscript. Cuiping Wu, Dongri Piao, and Wentao Yang conceived the study and contributed designing the study. Hai Jiang contributed designing the study, analysis and interpretation of data and reviewed the initial draft manuscript. All authors read and approved the final manuscript.

Conflict of Interest

All authors declare that they have no competing interest.

Funding Source

This study was funded by Major Infectious Diseases such as AIDS and Viral Hepatitis Prevention and Control technology major projects grants 2018ZX10101002.

Ethical Approval

The ethics committee of WYCH approved the study. Written informed consent has been obtained from all patients in accordance with the Declaration of Helsinki.

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