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Seroprevalence of Brucellosis in HIV-Infected Patients in Sanandaj, Iran

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Background: Infection with human immunodeficiency virus (HIV) leads to cellular immune deficiency and theoretically patients infected with HIV are susceptible to brucellosis.
Objectives: The current study aimed to determine brucellosis rate in the patients infected with HIV.
Patients and Methods: We included 89 HIV^+ patients from Sanandaj Consultation Center for Behavioral Diseases. Patients signed informed written consent before filling out the questionnaire. After serum collection, standard Wright tube, Coombs-Wright and 2ME-Wright tests were performed. Moreover, blood samples obtained from 502 individuals, who were not infected with HIV, were served as the control.
Results: The mean age of participants in the experimental and control groups were 33.31 ± 7.47 and 34.38 ± 11.29 years, respectively. In the Wright tube test for the HIV^+ group, 71 individuals (79.8%) did not have an antibody against *Brucella* spp., while 18 patients (20.2%) were positive for the antibody. According to the results of Wright tube test for the control group, 63 (12.5%) participants were positive for anti-*Brucella* antibody. The frequency of antibody against *Brucella* spp. in the HIV^+ group was significantly higher than that of the control group (P = 0.042).
Conclusions: HIV positive individuals in areas endemic for brucellosis must be investigated for the disease.

Keywords: HIV; Seroprevalence; Brucellosis

1. Background

Infection caused by various species of *Brucella* spp. in human is called brucellosis (1, 2). The prodromal phase of the disease is two to three weeks, but in some patients may last for several months (3, 4). Serological methods for brucellosis diagnosis are of great importance to evaluate the disease prevalence in endemic areas (5, 6). Infection with human immunodeficiency virus (HIV) leads to cellular and humoral immune deficiency, and the infected individuals become susceptible to various pathogens and opportunistic microbes (7, 8). The immune response effective against *Brucella* spp. relies upon cellular immune response (9-12). In HIV infected patients, as the disease progresses, the number of CD_4^+ cells decrease, which leads to cellular immune deficiency (13). In brucellosis, elimination of the bacteria from contaminated macrophages is very hard (14). Iran is one of the endemic countries for brucellosis (15). Moreover, brucellosis can be considered as a risk factor for HIV positive patients.

2. Objectives

The current study aimed to evaluate the frequency of the antibody against *Brucella* spp. in HIV positive patients in the West of Iran.

3. Patients and Methods

This study was carried out in cooperation with the Sanandaj Consultation Center for Behavioral Diseases. All patients and controls were informed about the study and signed the written consent; 89 HIV positive patients were included in the study, and for each patient a questionnaire was filled out. The questionnaire included demographic information, age, gender, CD_4^+ cell count, and duration of the infection since diagnosis of the disease. A 5 mL blood sample was obtained from each patient and serological tests to detect anti-*Brucella* antibodies were carried out for each sample. HIV infection was diagnosed using the enzyme-linked immunosorbent assay (ELISA) and then the

Implication for health policy/practice/research/medical education:
Survey of brucellosis is suggested for HIV-infected patients in endemic areas of brucellosis due to the compromised immune system.

results were confirmed by the Western Blot method. The number of CD_4^+ cells in the infected patients was obtained from their medical file. Since risky behaviors including abusing IV-drugs, was the main route of infection acquisition for most of the patients, co-infection of HBV (Diaplus, San Francisco, USA), HCV (Diaplus, San Francisco, USA), and HTLV-1 (Dima, Goettingen, Germany) were evaluated by the ELISA test (16-18).

In the Wright test, standard agglutination tube test was used. In this method, for each patient, 12 tubes were used, and the patient's serum was exposed to the *Brucella* antigen (Pasteur Institute, Tehran, Iran). The serum was then incubated at 37 °C for 48 hours and the results were recorded. Coombs-Wright test (Pasteur Institute, Tehran, Iran) was performed after the Wright test. To determine the pattern of anti-*Brucella* antibodies, 2ME Wright test (Pasteur Institute, Tehran, Iran) was used (17, 19). Furthermore, after obtaining the informed written consent, 5 mL blood samples were obtained from 502 normal individuals who were negative for HIV, HBV, HCV (Diaplus, San Francisco, USA) and HTLV-1(Dima, Goettingen, Germany) infections in ELISA evaluations. The serological tests of anti-*Brucella* antibodies were performed on serum samples of these individuals.

3.1. Statistical Analysis

SPSS software, version 12 was employed to analyze the data and the results were provided in a tabular format according to descriptive statistics. Qualitative and quantitative data were compared by t-test and Chi-square test, respectively. P values less than, or equal to 0.05 were considered statistically significant.

4. Results

The mean age of HIV^+ patients was 33.31 ± 7.47 years, while the mean age of the control group was 34.38 ± 11.29 years. The two groups were not significantly different in this respect ($P > 0.05$). The mean CD_4^+ cell count in patients infected with HIV was 803± 656.7 cell/μL (Min: 50 cell/μL, Max: 4546 cell/μL). The mean number of CD_4^+ cells in women was higher than that of men, but the difference was not statistically significant ($P = 0.6$) (Table 1). The mean number of CD_4^+ cells in HIV patients positive for anti-*Brucella* antibody was 711.67 ± 331.71 cell/μL, while the number for patients negative for anti-*Brucella* antibody was 827.30 ± 716.88 cell/μL. The HIV^+ patients positive and negative for anti-*Brucella* antibody were not significantly different in this respect ($P = 0.5$). In the HIV^+ group, 71 (79.8%) patients were negative for anti-*Brucella* antibody. Among the 18 patients who were positive for anti-*Brucella* antibody, three patients had high titers of the antibody. In the Coombs-Wright test, all patients were negative (Table 2).

In the normal control group, 439 individuals (87.5%) were negative for the antibody, and from the 63 individuals (12.5%) who were positive for the antibody, only one had an antibody titer of 1/40 and the others had a titer of 1/20. The prevalence of anti-*Brucella* antibody in the HIV^+ group was higher than that of the control group ($P = 0.042$). In the HIV^+ group, all the 18 patients who were positive for anti-*Brucella* antibody were male. While in the control group, from those who were positive for the antibody, 57 individuals were male and six were female.

Table 1. Demographic, Epidemiological and Clinical Characteristics of HIV^+ Patients With Positive Anti-*Brucella* Antibody

	No. (%)	Individuals With Positive Anti-*Brucella* Antibody, No. (%)
Gender		
Male	79 (88.8)	18 (100)
Female	10 (11.2)	-
Mean CD_4 cell count, Mean ± SD		
Male	790.73 ± 683	-
Female	904.4 ± 406.65	-
HBV [a]		
Positive	1 (1.13)	-
Negative	88 (98.87)	18 (100)
HCV [a]		
Positive	31 (34.8)	4 (22.2)
Negative	58 (65.2)	14 (77.8)
HTLV-1 [a]		
Positive	-	-
Negative	89 (100)	-

[a] Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; HTLV, human T lymphotropic virus-1.

Table 2. Frequency Distribution of Negative and Positive Titers of Wright, Coombs-Wright and 2ME Tests of HIV$^+$ Patients

Test Result	Tube Wright, No. (%)	Coombs-Wright, No. (%)	2ME, No. (%)
Negative	71 (79.8)	89 (100)	88 (98.9)
1:20	8 (9)	-	-
1:40	4 (4.5)	-	-
1:80	3 (3.4)	-	-
1:320	2 (2.2)	-	1 (1.1)
1:1280	1 (1.1)	-	-
Total	89 (100)	89 (100)	89 (100)

5. Discussion

The obtained results indicated that the prevalence of anti-*Brucella* antibody in the HIV$^+$ group was higher than that of the control group (P = 0.042). This is in accordance with the results obtained by Abdollahi et al. They reported that the frequency of brucellosis in HIV$^+$ patients, determined by serological methods, was significantly higher than that of the control group (P < 0.001) (20). However, in the current study, the frequency of anti-*Brucella* antibody in the HIV$^+$ group was 20.2%, while this rate was reported 73.3% in the study carried out by Abdollahi et al. The rates obtained in the two studies were significantly different. In HIV infection, CD_4^+ T cells become infected with HIV, while the activities of the virus and the immune response kill the infected cells. This leads to a reduction in population of the cells in the body, and consequently deficiency of cellular immunity (20). In the current study, although the mean number of CD_4^+ cells in HIV$^+$ patients, positive for anti-*Brucella* antibody, was lower than that of HIV$^+$ patients, negative for the antibody, yet the difference was not statistically significant (P = 0.5). In the study carried out by Abdollahi et al., no statistically significant relationship was observed between the mean number of CD_4^+ cells and the presence of anti-*Brucella* antibody in HIV-infected patients (P > 0.05). Previous studies reported that the prevalence of brucellosis in male HIV$^+$ patients was higher than that of female patients (20, 21). In the current study, anti-*Brucella* antibody was observed only in male HIV-infected patients. In developed countries where brucellosis is under control, the ratio of male to female *Brucella* spp. infection was reported 1:5 to 1:6 (21). In almost all reports from brucellosis endemic and non-endemic countries, the rate of infection in men is higher than that of women. The underlying causes have been considered to be occupational factors or contact with livestock (22, 23). Our previous study and other reports showed that brucellosis mostly occurs in people taking contaminated dairy products, or those who have direct exposure to infected livestock, which occurs in slaughterhouses and ranches (19, 24, 25). The prevalence of brucellosis is high in west of Iran. Therefore, high-risk individuals such as those who have weak immune systems or HIV$^+$ patients must be screened for brucellosis.

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Authors' Contribution

Study concept and design: Rezaee, Rahmani and Jalili; analysis and interpretation of data: Rezaee, Rashidi, Ghaedi, Pazoki and Menbari; drafting of the manuscript: Rezaee, Rahmani, Ghaedi and Jalili; critical revision of the manuscript for important intellectual content: Rezaee, Rahmani, Ghaedi, Jalili, Rashidi and Menbari; statistical analysis: Rezaee and Rahmani.

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The authors declare that there are no conflicts of interest.

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