Brucella Infection in HIV Infected Patients

Mahboubeh Hajiabdolbaghi, Maryam Rasoulinejad, Alireza Abdollahi, Koosha Paydary, Pegah Valiollahi, SeyedAhmad SeyedAlinaghi, Maziar Parsa, and Sirous Jafari

Iranian Research Center for HIV/AIDS (IRCHA), Tehran University of Medical Sciences, Tehran, Iran

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Abstract- The purpose of this study was to assess the possible correlation between *Brucella* and HIV infections. Iran is a country where HIV infection is expanding and Brucellosis is prevalent. In the present study, 184 HIV infected patients were assigned and for all of them HIV infection was confirmed by western blot test. In order to identify the prevalence rate of *Brucella* infection and systemic brucellosis in these subjects, sera samples were obtained and *Brucella* specific serological tests were performed to reveal antibody titers. Detailed history was taken and physical examination was carried out for all of patients. 11 (6%) subjects had high titers but only 3 of them were symptomatic. Most of these subjects were injection drug user (IDU) men and one was a rural woman. Considering both prevalence rates of *Brucella* infection (3%) and symptomatic brucellosis (0.1%) in Iran, our HIV positive patients show higher rates of *Brucella* infection and systemic brucellosis. Preserved cellular immunity of participants and retention of granulocytes activity may explain this poor association; whereas other explanations such as immunological state difference and non-overlapping geographical distribution of the 2 pathogens have been mentioned by various authors.

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Introduction

Systemic brucellosis is characterized by involvement of tissues rich in reticuloendothelial elements and profound activation of cell-mediated immunity (1-2). Similar to other zoonotic diseases, Iran is an endemic country for *Brucella* infection and symptomatic brucellosis (3). Among the affected populations, HIV infected patients might be at a greater risk for *Brucella* infection. The dramatic decline of CD4 marker level in HIV infected patients predisposes them to organisms that are mostly eradicated via cell-mediated immunity (4-6). Therefore, a frequent association could be anticipated within geographical areas in which both brucellosis and HIV are prevalent. Due to its unspecific and mistrustful clinical features, the prevalence rate of brucellosis is almost often underestimated in HIV positive population of *Brucella* endemic countries (7,8).

Within early 1990s, the possible association between brucellosis and HIV infection has been assessed only in a few endemic countries. To date, there have been only two evaluations of *Brucella* infection prevalence in hospitalized patients, most of which were asymptomatic HIV positive patients with a partially preserved immune system. Most of these studies have shown that there is no significant association between *Brucella* infection and the state of patient's immune system. These findings reveal no important correlation, although epidemiological facts highlight the need for further investigations (8,9). To the point, coincidence of *Brucella* and HIV infection has rarely been described in immunocompromised patients (10-12); but to prevent development of brucellosis complications, identification of clinical signs should be accompanied by serological tests in each HIV infected patient with unexplained clinical conditions, especially in *Brucella* endemic countries such as Iran (13-16).

Iran is a country which HIV infection is expanding and Brucellosis is prevalent. People living in rural areas are at a higher risk for acquisition of *Brucella* infection, while living in cities has a greater risk for exposure to human immunodeficiency virus. Therefore, we had the opportunity to investigate the association between the two infections. For the first time in Iran, we conducted this study to identify the prevalence rate of *Brucella* infection in HIV positive patients.

Corresponding Author: Sirous Jafari
Iranian Research Center for HIV/AIDS (IRCHA), Tehran University of Medical Sciences, Tehran, Iran.
Tel: +98 21 66947984, Fax: +98 21 66947984, E-mail: jafari_sirous@yahoo.com

Patients and Methods

This cross-sectional study was carried out in 184 HIV infected patients who had referred to the Voluntary Counseling and Testing (VCT) center of Imam Khomeini hospital, Tehran, from September 2007 to September 2008. Patients were consecutively assigned to participate and definite HIV infection was confirmed by performing western blot test for all of the subjects. After procedures and patient's privacy policy were explained to subjects, those who did not agree to participate for any reason were excluded before their enrolment. Informed written consent was filled out. This study was conducted with the approval of Institution Review Board (IRB) of Tehran University of Medical Sciences.

After blood samples were obtained from patients, standard serological tests including Wright, Coomb's wright, 2ME (2-mercaptoethanol) and ELISA (Enzyme-linked immunosorbent assay for IgM and IgG) were performed. In order to obtain demographic and therapeutic features (age, sex, taking highly active anti-retroviral therapy or HAART, CD4 marker level and duration of the HIV infection) questionnaires were filled out by all of the subjects. These questionnaires also provided us with information about past medical history and family history of brucellosis, brucellosis-related symptoms (e.g. fever, arthralgia, myalgia, etc) and history of raw dairy products consumption, history of direct contact with domestic animals, history of addiction, drug history of taking Trimethoprime-Sulfamethoxazole (Cotrimoxazole) in the last year and history of being prisoner in jail. In case of positive serology, liver function test results and other lab data including CBC, ESR (Erythrocyte Sedimentation Rate) and CRP (C-Reactive Protein) were obtained via patient's profile and attached to the questionnaires. Collected data were analyzed using the SPSS software.

Results

In this study we examined a sample of 184 HIV infected patients (149 men and 35 women) for *Brucella* serological tests; the mean age of patients was 37.6 years. 173 (94%) patients were city inhabitants and 11 (6%) patients were residents of rural areas. Of these, 126 (68.5%) patients were injection drug users (IDU) and 115 (62.5%) patients had a history of being in jail. Based on probable route of HIV transmission, patients were classified into different groups: 71 (38.7%) patients were IDU, 41 (22.3%) patients had a history of unprotected sexual contact while 56 (30.4%) patients were both IDU and a history of unprotected sex. Eight (4.3%) patients were infected by contaminated blood products and the remaining 8 (4.3%) patients were infected through undetermined routes of transmission. 117 (63.6%) patients have been taking HAART during the last year and the mean duration of HIV infection diagnosis was 3.2 years. Based on CDC criteria for clinical stages of the HIV infection, 62 (33.7%) subjects were in stage A, 65 (35.3%) subjects were in stage B and the rest of subjects (57 patients, 31%) were in stage C. The mean CD4 count was 320.2 cells/ml and in 31% of the patients CD4 count was less than 200 cells/ml. 45 patients had taken Cotrimoxazole in their drug history. Table shows the *Brucella* serology test results based on positive, suspicious and negative titers for each test.

Among the 184 sera samples obtained from subjects, serological markers were positive in only 11 (6%) patients, while titers were suspicious in 14 (7.6%) patients, and the remains (159 subjects, 86.4%) were reported as negative. Of these 11 patients, 10 were men and 1 was a rural woman. Most of these patients were IDU and had a history of imprisonment. The mean CD4 level was 235 cells/ml and 8 of them had not taken HAART during the last year. Four patients had a history of taking Cotrimoxazole and the mean duration of HIV infection was 3.1 years.

Table 1. Serological test results in 184 HIV infected patients.

	Wright	Coomb's wright	2-ME	ELISA	
				IgM	IgG
Positive	94.6% (≥1/40)	86.4% (≥1/40)	98.4% (≥1/40)	97.9% (≤8)	33.7% (≤8)
Suspicious	3.3% (1/80)	7.6% (1/80)	.....	1.6% (8≤titre≤12)	25.5% (8≤titre≤12)
Negative	2.1% (1/160≤)	6% (1/160≤)	1.6% (1/80≤)	0.5% (12≤)	40.8% (12≤)

Except the 3 patients for whom we had no evidence of antecedent *Brucella* infection, any positive family history of brucellosis or any data regarding exposure to infected animals or history of previously diagnosed brucellusis was only positive in 2 patients.

One patient was a 33 year-old rural woman who had a history of raw milk consumption and exposure to infected animals (e.g. sheep, goat, etc). She also had a positive family history of brucellosis and the HIV infection was diagnosed 9 years ago. This patient was in stage B of the infection and had taken both Cotrimoxazole and HAART during the last year. Her lab data included ESR=42 mm/h and CD4=166 cells/ml. Her physical examination revealed mild hepatosplenomegally, without any other localized sign or symptom. Another symptomatic patient was a 47 year-old IDU male with a history of unpasteurized milk consumption and a CD4 level equal to 124 cells/ml. He was in stage A and HIV infection was diagnosed 3 years ago. He had not taken HAART and his lab data were unremarkable.

The last symptomatic patient was a 29 year-old IDU male with a history of imprisonment and 3 years of HIV infection. He had not taken HAART during the last year; with a CD4 equal to 227 cells/ml, he was in stage B of the HIV infection. His serological test results are as follows: Coomb's Wright=1/640, 2ME=1/80 and Wright=1/640; although unfortunately he was lost to follow up during the course of study and we had no access to any address or number from him. Therefore, we were not able to document any past medical history of brucellosis in his family and possible risk factors of *Brucella* infection remained undetected. According to recorded data in his profile, diarrhea, arthromyalgia and pharyngitis were predominant symptoms. Except for a high inflammatory marker level (ESR=42 mm/h) he had no other remarkable lab data. Even though we did not follow these patients for a long period of time, no case of relapse, symptom recurrence or refractory infection was documented until the end of study, for a follow up period of approximately 12 months.

Discussion

In our study, antibody titers revealed *Brucella* infection in 6% of patients, most of which were asymptomatic and more than half of them had no previous epidemiological antecedent for *Brucella* infection acquisition. Despite the fact that eradication of intracellular *Brucella* is largely dependent on cell-mediated immunity, previous studies have revealed insignificant association between *Brucella* and HIV infection (17). Moreover, no predisposing immunological defect has been identified for *Brucella* infection. Thereby, one may assume that brucellosis is generally an infection of the immunocompetent. In this study, we have shown that both brucellosis and infection with *Brucella* species in HIV infected population are slightly higher than the normal population.

In a similar study in Kenya, Paul *et al.* (9) speculated that there is no significant difference between HIV positive and HIV negative subjects in vulnerability to *Brucella* as a potential intracellular parasite. A recent study in Iran showed a significantly higher *Brucella* serology in HIV-infected patients (18). In another study of Moreno *et al.* in Spain (8), they reported the characteristics of all HIV infected patients since the beginning of the AIDS epidemics, and in 12 patients, *Brucella* infection was diagnosed. Consistent with our study, most of the co-infected subjects were IDU men and presumed source of *Brucella* infection was identified for 11 patients. In our study, probable sources of infection were identified in only 2 patients, while 6 patients had a negative history and for 3 patients we had no accurate past medical history or any evidence regarding routes of *Brucella* transmission. In agreement with the study of spanish patients, biochemical analysis of our patients was unremarkable and physical examination revealed hepatosplenomegally in only one patient.

Based on epidemiological investigations in Iran, *Brucella* infection incidence has been reported 132/100000 (2,3); but recently, it has been argued that by taking the undiagnosed asymptomatic cases into account, the exact incidence rate is by far 25 folds more than the previous estimations (up to 3%). By assuming that the proportion of asymptomatic *Brucella* infection cases undiagnosed within this country is somewhat equal to asymptomatic *Brucella* and HIV infected subjects of our study, brucellosis prevalence is fairly higher (approximately 10 fold) in HIV positive patients. In fact, in only 2 subjects of our study *Brucella* infection developed to symptomatic brucellosis (~1%), while the rest (9 subjects) were asymptomatic. In regard to previous reports of symptomatic and thus diagnosed brucellosis incidence rate of the country (~0.1%), our data are inconsistent with previous studies. As a matter of fact, the prevalence rate of symptomatic brucellosis in our patient series is somewhat higher than those in normal population. Additionally, comparison of co-infected patients of our study (~6%) and estimated *Brucella* infection of the country (3%) clarifies the

higher rate of *Brucella* infection in HIV infected population. However, as far as we have no exact data regarding *Brucella* infection prevalence in Iran, it is not truthful to conclude that *Brucella* infection prevalence in HIV infected patients of this country is extraordinarily higher than HIV negative patients.

Preserved cellular immunity or initiation of prompt antiviral treatment shortly after the identification of HIV infection may eventually mask the real rate of *Brucella* infection in HIV infected. However, several additional explanations are likely to account for this insignificant association observed in studies done until today. Based on previous studies, it seems that HIV and *Brucella* co-infection does not result in the development of overt clinical properties of a classical systemic brucellosis. In our study, most of the co-infected patients did not mention troublesome symptoms nor had any signs of active brucellosis been revealed on physical examination (except for one case of hepatosplenomegally). Therefore, *Brucella* infection in HIV positive subjects of our study did not develop into active disease in at least 80% of the co-infected cases. One possible explanation is that granulocytes retain their activity during the very first stages of cell-mediated immunity deterioration. Regarding high incidence rates of *Brucella* infection in some parts of the world, very few infections develop into diseases, and most surprising, HIV positive and negative populations are slightly similar in the development of brucellosis complications. Furthermore, considering the epidemiological route of *Brucella* transmission, *Brucella* infection is a frequently observed clinical condition of rural areas. Urban areas, in contrast, have a very low incidence rate of zoonotic illnesses such as brucellosis. Consequently, an alternative explanation for insignificance of this suspected association would be modest exposure to both parasites: HIV infection has a higher prevalence in urban areas meanwhile *Brucella* is typically endemic in rural places. On the other hand, if this assumption of non-overlapping geographical distribution is true, one may expect higher rates of *Brucella* infection in the HIV infected patients of rural places. Within 11 rural patients of our study, only 1 was co-infected (~1%), which is fairly similar to that of city residents. Thereby, our observation argues this hypothesis of concurrent exposure. Moreover, for 6 of the co-infected subjects, no recorded epidemiological antecedent for acquisition of *Brucella* infection was evident. These patients were all city residents and IDU. It has been shown that infection with *Brucella* species promotes a cell-mediated immune reaction and induces the production of multiple cytokines, most important of which are IL-4 and IFN-γ. Among these pleiotropic cytokines, Th1 cytokines confer resistance, whereas Th2 cytokines predispose to brucellosis. Among significant cytokines during the immune reaction, IFN-γ plays a pivotal role in control of *Brucella* infection while IL-4 antagonizes its effects and inhibits cell-mediated immunity (18,19). A few studies suggest that immune reactions are probably crucial for the development of brucellosis from *Brucella* infection (20). Hence this immune response is phenotypically polymorphic in different cases with different immunological state, and the range of clinical manifestations widely varies among patients, one may assume that brucellosis features are likely correlated with the state of patient's immune system. Therefore, variable clinical responses to *Brucella* infection are expected in HIV patients with varying CD4+ levels. However, one may conclude that CD4+ count would be inversely correlated with the severity of brucellosis complications. In our study, no remarkable clinical finding was revealed nor was any worrisome symptom mentioned by any patients.

Major limitations of our study including data collection based on subjective questionnaires and patients profiles in combine with inaccessibility to patient's addresses or numbers; highlight the need for further investigations. For instance, detailed information of a *Brucella* infected subject with the highest titer levels has been unluckily lost during the course of study. Precise and explicit conclusions regarding brucellosis prevalence in HIV positive patients are reliable through prospective studies with larger sample sizes. Additionally, comparisons with HIV negative populations have to be assessed via considering a control group. A high degree of suspicion is needed to establish the diagnosis of brucellosis in HIV patients; besides, brucellosis clinical manifestations are basically unremarkable in HIV patients (21-24). Despite these limitations, our study shows that brucellosis has to be considered as a firmly significant differential diagnosis in any HIV positive patient with myalgia, fever or other unexplained conditions, particularly in brucellosis endemic regions of the world.

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