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Original Article

Investigation and Follow-up of Brucellosis in Seropositive Patients and Their Families in Hamadan Province, Iran

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Abstract

Background: Brucellosis is recognized as one of the most prevalent diseases among humans and animals. This study investigated and followed up brucellosis in seropositive participants in the Famenin (Hamadan province, Iran) cohort of brucellosis and their families by culture and serology methods.

Methods: Blood samples were taken from 66 subjects, including 18 subjects in the Famenin brucellosis cohort study with antibody titers ≥ 1:180 and 36 subjects from their families and 12 subjects in the Famenin brucellosis cohort study with antibody titers < 1:80. In the serological method, standard tube agglutination test (STAT positive with ≥ 1:80) and 2-mercaptoethanol (2-ME) test (positive with ≥ 1:40) were performed using the patient serum. Finally, 8 cc of the blood of all subjects was used for culture in the BACTEC culture medium.

Results: Of the 66 serum samples, 20 (30.3%) samples, including 5, 4, and 10 samples at 1:20, 1:40, and 1:80 dilution, respectively, and 1 sample at 1:160 dilution were positive by the STAT, of which 13 (65%) samples belonged to patients' family members. Using the 2-ME test, 10 (15.2%) serum samples were positive, of which 5 (50%) cases were related to patients' family members. Eventually, no growth of *Brucella* was observed in 66 flasks of the BACTEC culture medium.

Conclusion: Considering that a definite diagnostic method is not yet accessible, a combination of methods must be applied to diagnose the disease.

Keywords: Brucellosis, *Brucella melitensis*, *Brucella abortus*, Serology, Culture

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Introduction

Brucellosis is a global zoonosis caused by *Brucella* species that are Gram-negative facultative intracellular bacteria, which affect both humans and numerous animal species. According to the World Health Organization (WHO), approximately 500 000 new cases of human brucellosis are reported annually (1-3). Despite animal vaccination, brucellosis is endemic in some developing countries, including Iran (4,5). Brucellosis is characterized by a wide range of clinical manifestations and transmission from animals to humans in different ways (6). It is a significant cause of economic losses and veterinary health care costs and mortality in low-income countries (7,8). The clinical signs and symptoms suggestive of brucellosis are abortion, stillbirth, orchitis, arthritis in animals and undulant fever, anorexia, malaise, fatigue, weight loss, arthralgia, sweating, cough, nausea, and vomiting in humans. The timely and correct recognition of brucellosis is difficult due to the unspecific signs and symptoms of brucellosis (9). The disease diagnosis mostly relies on the presence of clinical signs and symptoms, together with epidemiological and serologic findings or identification of *Brucella* spp. from culture (10). Blood culture is the best method for laboratory brucellosis diagnosis. However, the proportion of positive cultures ranges from 15% to 85% (11,12). Although different serological tests, including the standard tube agglutination test or Wright test and 2-mercaptoethanol (2-ME) test, have been commonly used to diagnose brucellosis, the specificity of these tests is low, especially in endemic areas, due to the high prevalence of antibodies in the healthy population (13-15). Titers 1:160 or greater in the Wright test, which measures the immunoglobulin M (IgM) and IgG, and titers 1:40 or greater in the 2-ME test, which only measures IgG, should be considered as diagnostic for *Brucella* (10). *Brucella canis* should be considered in negative Wright results because it is rare in humans (16). It has been reported that decreasing in the level of IgG is an indicator of therapy success (14).

Therefore, this study sought to evaluate and follow up on brucellosis disease in seropositive participants in the Famenin cohort of brucellosis and their families using culture and serology methods.

Materials and Methods

Collection of Samples and Culture

The study was conducted during 2018-2020 in the comprehensive laboratory and microbiology laboratory of Hamadan University of Medical Sciences. After receiving approval from the Ethics Committee of Hamadan University of Medical Sciences and before taking the sample, informed consent was obtained from all patients. Blood samples were taken from 66 subjects, including 18 subjects in the Famenin brucellosis cohort study with antibody titers ≥ 1:180 and 36 subjects from their families and 12 subjects in the Famenin brucellosis cohort study with antibody titers < 1:180. Then, 10 mL of blood samples (8 mL of a blood sample for the BACTEC culture medium and 2 mL of a blood sample for serum isolation for serological tests) were taken from the subjects. Blood samples were cultured using a BACTEC automated blood culture system, and the serum samples were stored in a -20 ˚C freezer for Wright and 2-ME serological tests. Blood cultures were incubated in the BACTEC automated blood culture system for one week. Negative blood cultures were removed from the BACTEC system, incubated at 37 ˚C for another 3 weeks, and subcultured on blood agar and *Brucella* agar mediums at the end of each week. However, it should be noted that the negative blood culture media were incubated in the BACTEC system for 14 days.

Serological Tests

All clotted blood samples were centrifuged at 4000 rpm for 10 minutes, and the Wright and 2-ME serological tests were performed to detect *Brucella* antibodies. Antibody titers ≥ 1:80 and titers ≥ 1:40 were considered positive for Wright and 2-ME tests, respectively (17). Data were collected from standard questionnaires collected during the blood sampling process in the Famenin brucellosis cohort study.

Statistical Analysis

The obtained data, including descriptive statistics (frequency and percentage), were analyzed by SPSS software, version 16 (Chicago, IL, USA).

Results

Overall, 66 samples were used in this study, of whom 29 and 10 patients had a history of contact with livestock and infection, respectively. In addition, two cases were treated, and one had a history of relapse. The age range of patients was 8-88 years (mean 45.5 years), In general, 29 (43%) and 37 (57%) cases were men and women, respectively. Moreover, 20 (30.3%) out of 66 serum samples were serologically positive with the Wright test. Based on the results, 5, 4, and 10 sera had agglutinin levels of 1:20, 1:40, and 1:80, respectively, and one sample had a titer of 1:160 (Table 1). Out of 20 people with a positive Wright test result, 13 (65%) patients' family members had a positive result. Furthermore, 10 (15.2%) serum samples (with titers ≥ 1:40) were serologically positive with the 2-ME test, of which 5 (50%) cases were related to the family members of the patients (Table 2). No growth of *Brucella* was observed in 66 flasks of the BACTEC culture medium.

Discussion

Brucellosis is a common human-livestock bacterial disease, and its clinical manifestations are variable (18). Hamadan with an incidence of 81.4 per 100 000 people is one of the provinces with a high prevalence of brucellosis, and brucellosis in the nomadic and rural population of the Hamadan province had an increasing trend between 2008 and 2013. Consequently, the control of brucellosis in the nomadic and rural areas of Hamadan province is considered a health priority in the region (19). The basic diagnosis of brucellosis in humans is cultural and serological. The conclusive diagnosis of brucellosis is the isolation of *Brucella* from the blood, aspiration of the bone marrow, or body fluids in the case of infection of the affected organ. Nevertheless, its positivity shows a great variety (1,20). In the present study, no growth of *Brucella* was observed in 66 flasks of the BACTEC culture medium. Similar to our study, in the study of Sathyanarayan et al, none of the blood cultures isolated *Brucella* species using

Table 1. Break-up of the Agglutination Titre With the Wright Test and the Number of Cases in Each Group

Agglutination Titer	Number of Cases (%)
Negative	46 (69.71)
1:20	5 (7.57)
1:40	4 (6.06)
1:80	10 (15.15)
1:160	1 (1.51)
1:320	-
1:640	-
1:1280	-
1:2560	-

Table 2. Break-up of the Agglutination Titre With the 2-ME Test and the Number of Cases in Each Group

Agglutination Titer	Number of Cases (%)
Negative	56 (84.84)
1:20	-
1:40	5 (7.6)
1:80	2 (3.03)
1:160	-
1:320	1 (1.51)
1:640	1 (1.51)
1:1280	1 (1.51)
1:2560	-

Note. 2-ME: 2-Mercaptoethanol.

the Castaneda method (21), which is in line with the results reported by Joshi et al (22). This can be attributed to the experimental antibiotic treatment given to patients, which suppresses bacterial growth. Blood culture from *Brucella abortus* is also rare in brucellosis. None of the serological methods used to diagnose brucellosis have 100% sensitivity and specificity. In addition, in many cases, serological tests are associated with false-positive and negative results (20,23). Serology remains the mainstay of laboratory diagnosis, but the interpretation of results is full of difficulties, and the large number of applied techniques is evidence of these problems. Wright test measures IgM and IgG, and titers ≥ 1:80 are considered positive in Iran. It is important to note that the Wright test is positive in 97% of cases until the third week of the disease and remains positive for more than 2 years in 5-7% of cases after treatment. Occasionally, the phenomenon of Prozone occurs due to the high level of antibodies in the acute stage of the disease, which disappears by diluting the serum to 1:1280 (24). In the present study, the Wright and 2-ME tests were 30.3% and 15.2% positive, respectively. In this study, 13 (65%) and 5 (50%) of the positive results of the Wright and 2-ME tests belonged to patients' family members, respectively. In a study of 91 patients from Turkey, 84 (92%) cases demonstrated a serological titer of 1: 160 in the Wright test, and 28 (31%) patients had a positive blood culture (25). In the study of Dal et al, among 153 patients with suspected brucellosis, 36 (23.5%) and 88 (57.5%) cases had positive blood culture and Wright test, respectively (26). In the Pourakbari et al, blood and bone marrow cultures were 30% and 31% positive, respectively. Wright and 2-ME tests were also 67% and 85% positive, respectively (10). In the study of Torkaman Asadi et al, the Wright test, 2-ME, and culture were 88.6%, 88.5%, and 38.3% positive, respectively (27). Hosseini-Doost et al reported that among 42 positive serological samples of livestock, only 6 samples were positive by the culture method. Their study also revealed that out of five samples of positive human serology with the clinical signs of brucellosis, all were confirmed by culture (28).

Aminzadeh et al studied the family members and colleagues of brucellosis patients to diagnose the undiagnosed cases of brucellosis by enzyme-linked immunosorbent assay (ELISA). Positive IgM, IgA, and IgG titers by ELISA were observed in 6%, 21.5%, and 26.5% of the subjects, respectively. Serological prevalence and different clinical symptoms were observed in 40 (34.2%) and 38 (32.5%) people, respectively. By discovering the cases of the disease in patients' relatives, they concluded that due to the high prevalence of positive serology and symptomatic people among the relatives of the patients with brucellosis, it seems that the family members of the patients with brucellosis are not the only high-risk group, and diagnosis of the disease in other people around the patients, including their colleagues, is also necessary (29). Among the limitations of the present study were the low sample size and the lack of confirmation of the results with molecular methods such as the polymerase chain reaction. In addition, considering the high prevalence of positive serology and symptomatic people among the close relatives of patients with brucellosis, it seems that the family members of patients with brucellosis are not the only high-risk group, and it is necessary to diagnose the disease in other close relatives of patients, including their colleagues.

Conclusion

The signs and symptoms of human brucellosis are not specific and cannot be diagnosed solely based on clinical signs. In the case of brucellosis, a strong clinical suspicion with a positive serological test is usually a diagnosis. However, the isolation of the causative organism from blood or bone marrow samples is conclusive evidence of the disease. The isolation rate of bacteria from clinical specimens is extremely low, and the clinical symptoms of brucellosis, along with serological tests, can lead to a possible diagnosis of the disease. Unfortunately, a definitive diagnostic method is not yet available, thus a combination of methods must be used to diagnose the disease.

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Competing Interests

The authors declare no conflict of interests.

Ethical Approval

The Ethical Committee of Hamadan University of Medical Sciences approved the investigation (the ethical approval No: IR.UMSHA.REC.1397.647).

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