Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

Pharmacophore
an International Journal
ISSN 2229-5402

COMPARING CLINICAL AND SEROLOGICAL RESPONSES IN PATIENTS DIAGNOSED WITH BRUCELLOSIS

Mohamad Reza Nazer[1], Shahram Shokri [2*],Sanaz Asadi[3],Hosein Haji Amoo Asar[4]

1. Department of Infectious Diseases, Lorestan University of Medical Sciences, Khorramabad ,Iran

2. Department of Infectious Diseases,Lorestan University of Medical Sciences, Khorramabad ,Iran

3. General Physision, Lorestan University of Medical Sciences, Khorramaba , Iran

4. Department of Physical Medicine & Rehabilitation, Lorestan University of Medical Sciences, Khorrama , Iran

ARTICLE INFO

Received:
03th Jun 2017
Accepted:
29th Nov 2017
Available online:
14th Dec 2017

Keywords: *brucellosis, clinical symptoms, serology tests, zoonosis*

ABSTRACT

Introduction: Brucellosis is a bacterial disease common between the humans and animals and it is highly prevalent all around the globe and Lorestan Province has been recognized as one of the endemic regions for the foresaid disease. The disease reportedly has a high prevalence rate in Iran. The clinical symptoms of the disease are very diverse in such a manner that 26 cases are left undiagnosed per every well-justified brucellosis case. Thus, according to the importance of this native disease and its clinical diversity as well as for the problems residing in its diagnosis and treatment, the current research paper aims at comparing the improvement of symptoms and serological titers of the disease as well as figuring out any relationship between them.

Method: the study is a retrospective cross-sectional research that has been carried out based on census method on all the patients with brucellosis who have referred to infectious diseases clinic in Shohaday-e-Ashayer Hospital in 2012. The patients who were found having the required perfect information corresponding to a checklist were allowed to enter the study and their clinical symptoms were compared in serological tests in different time intervals, namely the onset of the treatment, two months and four months after the onset of the treatment.

Findings: Out of the 70 patients studied herein 31 (44.3%) were female and 39 (55.7%) were male. At the beginning of the treatment, 88.6% of the patients had backache as well as musculoskeletal pains; 80% had fatigue and debility; 58.6% had headache and 50% had fever. Two months after the initiation of treatment, the clinical symptoms of the patients in an order of frequency were fatigue and debility, backache and musculoskeletal pains, headache and fever with the frequency rates of 51.4%, 34.3% and 12.9%, respectively. Four months after the onset of the treatments, the clinical symptoms by an order of frequency were fatigue and debility, headache, backache and musculoskeletal pains as well as fever with the frequency rates of 42.9%, 18.6%, 15.7% and 7.1%, respectively.

Conclusion: there was found a significant relationship between the serology tethers and patients' symptoms from two months since the onset of the treatment. Also, there was found a significant relationship between the clinical symptoms and Wright's test in five of the cases, Wright's Coombs test in three cases and 2ME test in six cases and the relationship was also found nearly significant in two of the cases (P-value=0.006). According to the abovementioned results, the relationship was found statistically more significant between 2ME test and the clinical symptoms two months and four months after the onset of the treatment in contrast to Wright's test and Wright's Coombs test and this is indicative of the higher value of 2ME test in follow-up periods and figuring out the responses to the disease treatment.

To Cite This Article: Mohamad Reza Nazer , Shahram Shokri * ,Sanaz Asadi ,Hosein Haji Amoo Asar*, (2017), "Comparing Clinical and Serological Responses in Patients Diagnosed with Brucellosis", ***Pharmacophore, 8(6S),*** *e-1173873.*

Introduction

Brucellosis is an infectious disease that is transmitted by brucella genus microbes in animals and causes disease in human beings [1]. Such bacteria are more frequently found transmitted to the humans via nutrition and/or being in touch with the

Corresponding Author: Shahram Shokri, Department of Infectious Diseases,Lorestan University of Medical Sciences, Khorramabad ,Iran

infected animals or their secretions and they are considered as potential biological warfare and bioterrorism should be the first thought striking the mind in case of abnormal increase in disease cases in a region [2].

The disease is commonly termed undulant fever due to the rising and falling fever cycles. Except for a few countries that have completely eradicated the disease among their reservoir animals, the disease enjoys a global scattering. Although brucellosis manifests itself in the form of an acute fever, it has diverse clinical expressions and the decisive symptoms that lead to the diagnosis of the disease might be absent. Due to the same reason, the clinical diagnosis of the disease should be usually confirmed in bacteriological or serological examinations [3].

Annually, 500 thousand patients are found with brucellosis worldwide and some authors reckon that there is left 26 undiagnosed cases per every well-documented case of brucellosis [4].

The disease is usually transmitted via direct contact with the infected animal or through drinking infected milk or unpasteurized milk products as well as to a lesser degree through infected meat [5]; also, it has been reported to have been transmitted via inhaling the aerosols containing organism in laboratories and in places where the animals with the brucella bacteria are kept. It is also used against the humans like HIV and Hepatitis E as a weapon (bioterrorism) [6, 7]. The spattering of infected secretions to the conjunctiva and the piercing of the body by the infected needle during animal vaccination and/or in laboratories are also the other causes of human infection with the disease. The minimum infecting oral and inhalation dosages of melitensis are 5000 and 1300 organisms, respectively [8]. Based on the statistics issued by contagious disease prevention and control office, about 50 thousand cases of brucellosis are reported annually and the infections are in a range of 3.5 to 10.5 thousand cases in various provinces in the country including in Markazi Province, Lorestan Province, Yazd Province [9], Chaharmahal and Bakhtiyary Province' moreover, the lowest prevalence rate, below 0.5 per thousand, has been reported in Tehran, Kurdistan, Gilan and Zanjan provinces. Also, the other country's provinces have been reported to have a prevalence rate in between the two foresaid values. Brucellosis is considered a native disease in Iran and it is caused essentially by Brucella Melitensis [5].

Brucella separation is the most decisive method of brucellosis diagnosis. Blood, lymphocyte and bone marrow cultures are the best diagnostic methods. These methods have such disadvantages as being time-consuming, the risk of personnel infection and high rate of false negative responses [10, 11]. Wright's standard tube examination is more widely applied for the brucellosis diagnosis and it is known to agglutinate antibodies of IgM and IgG types and the test results are sometimes found falsely negative due to the presence of blocking antibodies in which case Wright's Coombs test or Wright's test in higher dilution rates can be applied.

In case of the disease recurrence or in case that no improvement is attained, IgG becomes positive which is reflective of the disease being in its active phase. In Wright's test, the Immunoglobulin, IgM, antigen can be eliminated assisted by 2-mercaptoethanol (2-ME) test and then the agglutination that occurs subsequently pertains to IgG. In the early onset of the disease that there is only IgM existent, Wright's test shows positive results but the 2-mercaptoethanol (2-ME) test might be found demonstrating negative results which is suggestive of the existence of a newly emergent and recent infection. ELISA test determines various IgG, IgA and IgM antibodies and it possesses a high sensitivity and specificity [12].

But, in Iran, as it is emphasized by Brucellosis committee of the country and world health organization, there is a need for paying more attention to the lower titers such as Wright's 1/80 and 2-ME in individuals with the prediagnosed with certain symptoms. The clinical symptoms of Brucellosis are nonspecific and include fever, sweating, dullness, anorexia, headache, backache and depression. Brucellosis is a systematic disease that can engage any organ or system of the body [13, 15].

The present study intends to perform a comparative study of the clinical and serological responses of brucellosis-prediagnosed patients under treatment in Khurram Abad (a city in west Iran) due to the high prevalence rate of brucellosis therein.

Study Method

The current study is a retrospective cross-sectional research. It evaluates the entire under-treatment patients with brucellosis who had referred to the infectious diseases clinic in Shohaday-e-Ashayer Hospital within a one-year cross-section based on a census research method. The study inclusion criterion was the study of the medical files of the patients who found having the complete information required for the study based on a previously-prepared checklist and the imperfect files were excluded.

The study tool was a researcher-constructed checklist containing 1) demographic information including age, gender and the season in which they had been inflicted with the disease as well as 2) the information pertaining to the serological tests including Wright's test, Wright's Coombs test and 2-ME test by an order of time for the onset of the treatment, two months after the onset of treatment and four months after the onset of the treatment and 3) the information pertaining to the clinical symptoms including the presence or absence of fever, headache, pain in muscles, backache, fatigue and debility separated and classified for the onset of treatment, two months after the onset of treatment and four months after the onset of treatment.

First of all, the author attended Shohaday-e-Ashayer hospital and then acquired the information required for the present study through referring to the medical files of the patients who had referred to the hospital for the treatment of brucellosis. The extracted information was inserted to the checklists and it was found out that the patient-reported clinical symptoms could not be attributed to any other disease than brucellosis. The information was collected and entered into SPSS software after which statistical tests were undertaken. The expressed clinical symptoms and serological tests were investigated based

on the aforesaid time order. Also, the clinical symptoms (fever, headache, fatigue, debility, pain in joints and backache) were compared with the serological tests' titers, Wright's Coombs test and 2-ME test based on a time order for the onset of treatment, two months after the onset of treatment and four months after the onset of treatment.

Findings

The present retrospective cross-sectional study was conducted on 70 under-treatment patients with brucellosis. Out of the 70 patients, 31 patients (44.3%) were female and 39 patients (55.7%) were male; also, 17 patients were below 20 years of age, 25 patients were between 20 and 29 years of age, 14 patients were between 30 and 39 years of age, 9 patients were between 40 and 49 years of age and 5 patients were above 50 years of age. In terms of the disease outbreak season, 29 patients had been inflicted with the disease in Spring, 26 patients in summer, 8 patients in fall and 7 patients had been diagnosed with the disease in winter (table 1).

In the current study, various clinical symptoms had been found in the patients, including fever, headache, musculoskeletal pains, fatigue and debility in the first appearance of the disease, two months and four months after the onset of the disease in such a manner that 88.6% of the patients complained of backache and musculoskeletal pains during the early days of the disease occurrence; 80% of the patients complained about fatigue and debility, 58.6% of the patients complained about headache and 50% of the patients complained about fever. The most prevalent clinical symptoms of the patients in the advent of the disease were backache and musculoskeletal diseases. The lowest prevalence rate belonged to fever, inter alia the aforementioned clinical symptoms, in the early days of the disease occurrence.

About two months since the onset of the disease, the patients' positive clinical symptoms by an order of prevalence were fatigue and debility, backache and musculoskeletal pains, headache and fever with 51.4%, 34.3% and 12.9%, respectively.

About four months after the onset of the treatment, the patients' positive clinical symptoms by an order of prevalence were fatigue and debility, headache, backache and musculoskeletal pains and fever with the frequency rates of 42.9%, 18.6%, 15.7% and 7.1%, respectively (Table 2).

The serological titers of the patients were investigated at the beginning of the treatment, after two months since the onset of treatment and after four months of the onset of treatment. The median, mode, inter-quartile range and minimum and maximum data are presented in table (3) in great details.

The clinical symptoms' relationship with the patients' serological titers was investigated at the beginning of the treatment, two months after the onset of treatment and four months after the treatment. The results of Mann-Whitney test signify the following cases: there was not found any significant relationship between Wright's titer test and the presence of fever (p-value=0.340); there was not found any significant relationship between Wright's Coombs test and the presence of fever (p-value= 0.361); there was not found any significant relationship between 2-ME test and the presence of fever; there was not found any significant relationship between the presence of headache and Wright's test (p-value=0.265); there was not found any significant relationship between the presence of headache and Wright's Coombs test (P-value=0.347); there was not found any significant relationship between headache and 2-ME test (P-value=0.269); there was not found any significant relationship between backache and musculoskeletal pains with Wright's test (p-value=0.938); there was not found any significant relationship between backache and musculoskeletal pains with Wright's Coombs test (p-value=0.923); there was not found any significant relationship between backache and musculoskeletal pains with 2-ME test (p-value=0.434); there was not found any significant relationship between the presence of fatigue and debility with Wright's test (p-value=0.239); there was not found any significant relationship between the presence of fatigue and debility and Wright's Coombs test (p-value=0.059); and, there was not found any significant relationship between the presence of fatigue and debility with 2-ME test (p-value=0.063).

The relationship between the clinical symptoms and serological titers was also evaluated two months after the onset of treatment. The results of Mann-Whitney test indicated the following cases: there was not found any significant relationship between Wright's titer test and the presence of fever within two months after the onset of treatment (p-value=0.312); there was not found any significant relationship between Wright's Coombs test and the presence of fever (p-value=0.021); there was found a significant relationship between 2-ME test and the presence of fever (p-value=0.005); there was not found any significant relationship between the presence of headache and Wright's test (p-value=0.015); there was not found any significant relationship between the presence of headache and 2-ME test but the computed value is very close to a significant value (p-value=0.006); there was found a significant relationship between backache and musculoskeletal pains and Wright's test (p-value=0.00); there was not found any significant relationship between the presence of backache and musculoskeletal pains and Wright's Coombs test (p-value=0.037); there was found a significant relationship between the presence of backache and musculoskeletal pains with 2-ME test (p-value=0.002); there was found a significant relationship between the presence of fatigue and debility with Wright's test (p-value=0.002); there was found a significant relationship between the presence of fatigue and debility with Wright's Coombs test (p-value=0.003); and, there was found a significant relationship between the presence of fatigue and debility with 2-ME test (p-value=0).

The relationship between the clinical symptoms and serological titers was evaluated in two months after the onset of treatment. The results of Mann-Whitney test are as stated in the following sentences: in two months after the onset of the treatment, there was not found any significant relationship between Wright's titer test and the presence of fever (p-

value=0.015); there was not found any significant relationship between Wright's Coombs test and the presence of fever (p-value=0.241); there was not found any significant relationship between 2-ME test and the presence of fever but the value obtained is very close to the significance value (p-value=0.006); there was not found any significant relationship between the presence of headache and Wright's test (p-value=0.015); there was found a significant relationship between the presence of headache and Wright's Coombs test (p-value=0.003); there was not found any significant relationship between the presence of headache and 2-ME test (p-value=0.177); there was found a significant relationship between the presence of backache and musculoskeletal pains with Wright's test (p-value=0.001); there was found a significant relationship between the presence of backache and musculoskeletal pains and Wright's Coombs test (p-value=0.003); there was found a significant relationship between the presence of backache and musculoskeletal pains with 2-ME test (p-value=0.000); there was found a significant relationship between the presence of fatigue and debility and Wright's test (p-value=0.004); there was found a significant relationship between the presence of fatigue and debility with Wright's Coombs test (p-value=0.005); there was found a significant relationship between the presence of fatigue and debility with 2-ME test (p-value=0.001).

Discussion and Conclusion

The current retrospective cross-sectional research has been carried out on 70 under-treatment patients with Brucellosis who had referred to infectious diseases clinic in Shohaday-e-Ashayer Hospital. The highest prevalence rate of the disease among the studied patients belonged to the 20 to 29 age range (25.7%) and the lowest prevalence rate of the disease belonged to the above 50 age range (7.1%). Also, the most prevalent seasons of the disease occurrence were spring and summer with rates equal to 41.4% and 37.1%, respectively.

In a 16-year prospective research conducted by Arizai et al in 2000 on 530 patients with the objective of recognizing the clinical symptoms and risk factors of infection recurrence, it was found out that the clinical expressions and the serological tests in recurrence cases are weaker as compared to the preliminary disease. Also, the risk factors that expose the patient to infection recurrence outlined in the aforesaid study were inappropriate antibiotic treatment, positive blood culture during the early occurrence of the disease or ten days before the initial infection, male gender and platelets below 150 thousand. Also, the other risk factors like the intensity of the disease and immune deficiency were found effective on the disease recurrence [16].

In another prospective research carried out by Mantin Moreno et al in 2001 on 105 Spanish patients, 11 out of the 105 patients were found with the recurrence of the disease. The clinical signs and serological titers and blood cultures of the treated patients were compared post-treatment with those of the patients with the recurrence of the disease.

It was demonstrated in the current research paper that blood culture and special symptoms like long fever, spondylitis, joint inflammation and/or epididymo-orchitis are the best and the most widely applied cases for the diagnosis of the brucellosis recurrence.

The existence of unclear symptoms during the first month after the treatment onset and the elevation in serology titers (for instance a chance ration of 1:7) is of a low help to the recognition of the disease recurrence in the course of the treatment; whereas, the elevation in serology titers up to several months post-treatment onset can be of value for making decisions regarding the treatment interventions [17].

In a study that was conducted in Saudi Arabia by Memish et al in 2002 on 68 patients with brucellosis whose blood culture posits positive results and in comparison to the results obtained for a 70-individual evidence group, a sensitivity and specificity of 95.6% and 100% were reported, respectively, in a standard agglutination test for brucellosis [18].

In a study performed by Mansoureh Heravi et al in 2001 on 380 patients in the city of Kashan, it was made clear that fever and backache have been the most common expressions of the hospitalized patients and according to the positive Wright's test in the majority of the patients it can be concluded that the foresaid test can be applied as a sensitive and reliable test as the first diagnostic step [19].

In another study that was undertaken in 2000 by Martin Morino et al with the title of "brucellosis after treatment: the diagnosis of its non-persistence", the following results were obtained. The study examined 105 patients out of whom eleven individuals were found with the disease recurrence. The post-treatment signs, serology titers and the results obtained from the improved inter-patient cultures were compared with the same characteristics of the ones who had been found with the disease recurrence. It was proposed in the foresaid study that the blood culture and the disease-specific symptoms such as elongated fever, spondylitis, epididymo-orchitis and arthritis are the most valuable data for the recognition of brucellosis recurrence. The existence of ambiguous signs following treatment, especially during the first month since the initiation of treatment, is of a lower value for the diagnosis [20].

In another study that was conducted in 2005 by Tim Buchanan et al, with the title of "2-ME, the appropriate tool for the prediction of Brucellosis improvement", the following results were attained. The foresaid study was carried out on 92 patients with an eighteen-month period of brucellosis. During the study, the patients were subjected to SAT and 2-ME tests and it was figured out that SAT (more than 1/160 titer) has remained positive for a period of 1.5 years in 44 patients, out of 99 subjects, with brucellosis (248%), in spite of sufficient antibiotic treatment. 2-ME test in 8 cases out of the 92 patients (9%) remained positive after the elapse of one year (titer above 1/160) and only in four cases out of the 92 study subjects, it remained positive for 1.5 years. None of the 84 patients whose 2-ME titer was found negative after one year did not have

any sign of brucellosis or advanced chronic brucellosis. On the contrary, 4 cases out of the 8 patients whose 2-ME titer was found appropriate after one year since the onset of antibiotic treatment still had persistent signs of brucellosis that marked their need for the continuation of antibiotic treatment. It was made clear in the aforementioned study that 2-ME test, as compared to SAT, is more preferable in terms of determining the adequacy of antibiotic treatment; also, positive 2-ME test is a stronger indicator in terms of chronic brucellosis recognition [21].

Furthermore, in a review study that was carried out by Wafa Al-Nassir in 2001, it was stated that after the termination of treatment term, there is a need for an examination to detect the focal point of a septic lesion following the persistence or recurrence of brucellosis symptoms. Also, serological tests and ELISA method can be used for the evaluation of the treatment response of the patients [22].

In another study that was conducted by Pilar Morato with the title of "follow-up period of the patients with brucellosis", on 30 under-treatment patients, it was posited that the serological methods, although being easier and faster, display high titer values for even longer periods after the treatment of brucellosis and despite the negative blood cultures and/or the absence of clinical evidences. IT was also proposed therein that some authors have opined the helpfulness of the higher serological and/or IgG titers for the diagnosis of brucellosis recurrence. However, at the time the disease symptoms become clearly detectable, only 40% of the patients exhibit clear increase in serological titers in contrast to the past results in cases of disease recurrence. In some of the patients, the serological level variations occur about three months since the emergence of the first recurrence symptoms and they are hence clinically not of a value [23].

In another study that was conducted in 2006 by G. Mantur et al on 2698 patients (above 15 years of age), in addition to an array of other results it was also found out that at the same time with the clinical improvement of the patients there is a descending trend of 2-ME titer in 77.79% of the patients but SAT remained positive even long after appropriate and sufficient antimicrobial treatments. Also, it was posited in the foresaid study that fever has been the only clinically clear sign and the only cause of the patients' complaint. Also, in the follow-up period of the studied patients, it was determined that 2-ME test becomes positive during the early period of the disease recurrence. Finally, it was suggested in the aforementioned study that in the world's endemic spots, 2-ME test is applied for the detection of the disease recurrence.

According to the results presented in the current research paper, there is a significant relationship between serological titers and the disease symptoms since two months after the initiation of the treatment. Also, a significant relationship was found between the abovementioned clinical symptoms and Wright's test in five of the cases that included muscular and skeletal backaches within two months post the initiation of treatment, fatigue and debility within two months since the onset of treatment, headache, backache and fatigue and debility within four months of the initiation of treatment.

Acknowledgement:

The author hereby wishes to express thankfulness to the entire reputable personnel of infectious diseases clinics in Shohaday-e-Ashayer Hospital as well as to the clinical research units of the same hospital.

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Table 1. disease frequency based on the studied variables

Variable		**Frequency (percentage)**
Gender	Female	31 (44.3)
	Male	39 (55.7)
Age group	Below 20	17 (24.3)
	20 to 29 years of age	25 (35.7)
	29 to 39 years of age	14 (20)
	40 to 49 years of age	9 (12.9)
	Above 50	5 (7.1)
The season of disease occurrence	Spring	29 (41.4)
	Summer	26 (37.1)
	Fall	8 (11.4)
	Winter	7(10)

Table 2. frequency and percentage of patients' positive symptoms in the beginning of, two month after and four month after the onset of treatment

Symptom examination time												
	The onset of treatment				Two months after the onset of treatment				Four months after the onset of treatment			
Type of the clinical symptoms	Fever	Headache	Backache and muscular and skeletal pains	Fatigue and debility	Fever	Headache	Backache and muscular and skeletal pains	Fatigue and debility	Fever	Headache	Backache and muscular and skeletal pains	Fatigue and debility
Frequency	35	41	62	56	9	22	24	36	5	13	11	30
Percentage	50	58.6	88.6	80	12.9	31.4	34.3	51.4	7.1	18.6	15.7	42.9

Table 3. describing the patients serological titers in the beginning of, after two months and after four month of the onset of treatment

Type of the test		**Wright's test**			**Wright's Coombs test**			**2-Me test**		
Test time		The onset of treatment	Two months post treatment	Four months post treatment	The onset of treatment	Two months post treatment	Four months post treatment	The onset of treatment	Two months post treatment	Four months post treatment
STATISTICAL INDEX	Exponent	1:320	1: 160	1:80	1:160	1:160	1:80	1: 40	1:40	1:40
	Median	1:320	1: 160	1:80	1: 240	1:160	1:80	1:80	1:60	1:40
	Inter-quartile range	0	160	80	80	160	20	0	40	40
	Minimum	1:80	1:20	1:20	1:40	1:20	1:20	1:20	1:20	1:20
	maximum	1:5120	1:1280	1:1280	1:5120	1:1280	1:640	1:2560	1:640	1:640