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Brucellosis Antibody Level of Hospitalized Patients in Hamadan, Western Iran.

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Abstract:

Diagnosis of brucellosis is based on the rises of antibody titer in patient sera. It is reported clinicians are frequently complain on some of serology results of brucellosis. In this research, all brucellosis patients who were confirmed clinically and by serological methods are entered to the study. In total, sera of 758 patients of Hamedan teaching hospitals were analyzed with Wright, Coombs and 2Mercapto-Ethanol tests. All these patients were received treatment after final diagnosis.

In the studied group 29 (3.82%) and 30 (3.95%) of patients had no antibody rise in Wright and 2ME respectively. One patient had antibody titer less than 1/40 in Coombs test.

Specific antibody level higher than 1/160 was observed in 49.31% of patients for the Wright test, while it was 43.46% in patients with antibody titer higher than 1/80 of 2ME test.

Conclusion: It seems some of those serology negative patients may involved with brucellosis and possibility of infection should be always considered in those serology negative patients since B. abortus antigen are applied in all serology tests. Analyzed results also indicate application of Coombs test can provide clear understanding for those cases with low antibody rises.

Key Words: Brucellosis, Serological diagnosis, Wright, Coombs Wright, 2ME.

Introduction:

Among infectious illnesses Brucellosis is one of the diseases that its diagnosis demands epidemiology, clinical, and laboratory information. At present, laboratories by themselves are not able to diagnose the infection with more confidence. Isolation and identification are the more assured methods for the diagnoses. However, many difficulties have reduced the sensitivity of these methods and they are not being used in many laboratories [1,3]. Studies have shown that good chances of isolation bacteria from blood cultures is less than 3% in Iran [4]. Isolation from bone marrow and liver tissues are easy, however, invasiveness of the procedures have caused less use of these samples by laboratories. Therefore, the attention is focused on serology tests.

WHO Brucellosis committee has introduced five tests including Rose-bengal, 2ME, Wright, Coombs, and Complement fixation as international standard methods. In addition, it has recommended to use of at least three tests for every serum samples [5]. Time of sampling is very important in serology diagnosis. IgM usually appears from days 5 to 7 and during next 2-3 weeks its titer will be at the highest rate. IgG appearance starts from days 14 to 21 and during next 2-3 weeks it will be on the maximum rate with more stable form. It even rise to 8 fold or more in 80% of the cases. In those patients with complete treatment, specific antibody will drop or clear within 6 months to one year after antibiotic therapy period. IgG will remain on high titer in those uncompleted treatment and chronic cases.

Specific antibody titers of people living in endemic parts are more than normal level and this can be problematic in diagnosis [6,7]. Clinical observations have indicated that antibody titer in many of the patients with Brucellosis is not high [8-10]. It is reported also antibody titer of the Wright test is less than 1:160 in 13-25% of cases with blood culture positive cases.

In this research, we have tried to analysis the serology results of inpatients in the Hamedan hospitals to interpret and to evaluate the antibody titers and the accuracy of the reported results by comparing results of three methods, since there is no known reported antibody titer in studied population.

Materials and Methods:

Study design: In the present descriptive work, 809 patients were diagnosed to have Brucellosis in Hamadan's hospitals during 5 years. There were 51 cases with no serological results for any of three tests (Wright, Coombs, and 2ME). Therefore these patients excluded from the study, unless they respond to the treatment.

Laboratory diagnostic Criteria: All these patients had positive serological tests with standard tube agglutination tests (STA) (The kit was provided from Pasteur institute (Iran) and detecting antibody titer equal or over than 1/80 was considered as positive serology test). According to the recommendation of La-

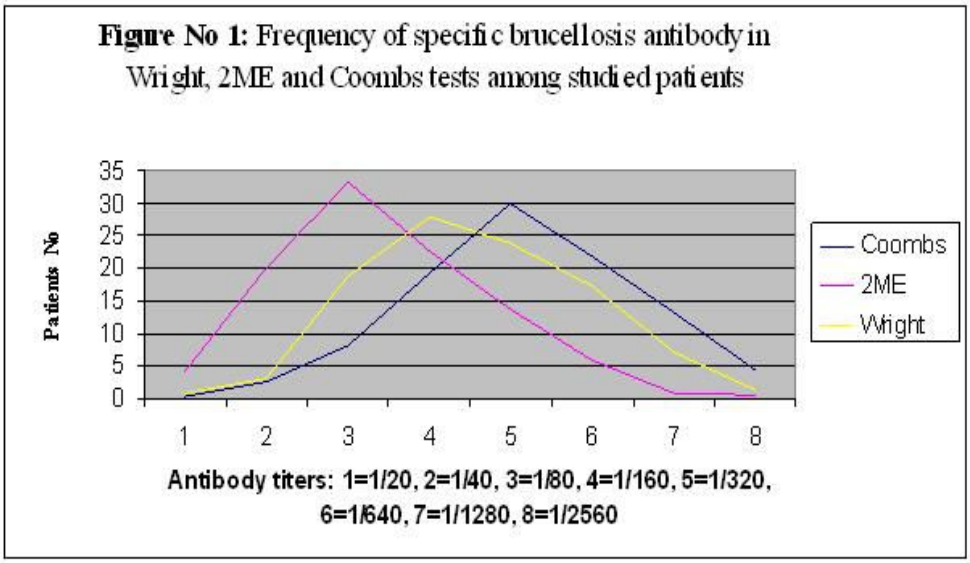
boratory Affairs Office of Iran, we considered 1:80 as a positive brucella titer for tube agglutination test. This test was applied and carried out on the basis of Razi protocol kit. Also 1:80 of 2ME test titer or more were chosen as a positive titer for brucellosis. In addition, 1:40 of Coombs specific antibody was considered as a positive result for those cases with the negative Wright and 2ME results. These tests were repeated after a while in some uncertain cases to ensure of rising antibodies.

Results:

Patients with negative results to one of the either tests: Analysis of the results revealed some of those diagnosed brucellosis patients had no positive antibody titer at the first time of admission, to the one of the either tests. The number of these patients varies for each test and is as follows: Number of patients with lower antibody titer for 2ME was 112 cases (23.89%), while it was 29 cases (3.82%) for Wright test. However there were only one patient with negative result for Coombs test (0.13%). This patient that had negative results for all three tests had responded to the treatment. (Table 1 & figure 1).

Table No. 1: Frequency of antibody titer in three serologic methods: Wright, 2ME, Coombs

Antibody titer	Wright (%)	2ME (%)	Coombs (%)
1/20	0.66	3.95	0.13
1/40	3.16	19.94	2.59
1/80	18.69	33.33	8.21
1/160	27.83	22.64	19.17
1/320	23.74	13.62	29.94
1/640	17.28	5.87	21.91
1/1280	6.99	0.83	13.11
1/2560	1.3	0.5	4.25
Total	100	100	100



Highest rate of antibody titer in the studied group:

The highest frequency of the antibody titer in the studied patients was different for each test:

Tube agglutination test: In this studied group, 27.83% patients with 1:160 of Wright antibody titer had the highest rate.

2ME test: Highest frequency rate of 2ME test was observed at 1:80 who were 33.33%

Coombs test: The highest number of patients who had positive Coombs results will observed in those results with at 1:320 titer and they were 29.94% of the patients.

Comparison the rate of Wright positive results with 2ME: Antibody level higher than 1/160 was observed in 49.31% of cases for Wright, while it was 20.82% in 2ME for patients having higher than 1/160 antibody level.

Discussion:

In Brucellosis IgM, IgG, IgA and small amount of IgE immunoglobulin will be secreted in the serum when humeral immunity is stimulated. After 5 to 7 days of infection, IgM is produced and during next 2 to 3 weeks will be in its peak. IgG is produced 2 to 3 weeks after infection and will be in its maximum peak in next 2 to 3 weeks. Therefore, if the serum samples are analyzed in the first week, the serology tests will be negative or it may show low titers. In this case Coombs test provide better discrimination since it has higher sensitivity.

If the serum sample is obtained in the second week, IgM is the predominant produced antibody. IgG production is gradually started from second and the third week. It will be in the highest amount and will have the predominant role after 2-3 weeks.

In this study all patients were under careful supervision because of their severe signs and symptoms. Therefore it would be expectable to have evidence of the specific antibodies. On the basis of analyzed results, 3.81%, 23.89% and 0.19% of patients had no expectable titer in Wright, 2ME and Coombs respectively. It seems apply Coombs test can underline better explanation for those cases with false negative results [6].

Those patients with acute or subacute brucellosis are expected to have antibody titer 1/160 for Wright and 1/80 for 2ME, while in chronic brucellosis, it is expected both Wright and 2ME tests to be 1/160. In the present study, 22.5% and 23.92% of the patients had lower antibody level than 1/160 and 1/80 in Wright and 2ME test respectively. In other words, 78.5% of the brucellosis patients had just Wright test with 1/160 titer and over. In another research, Amirzargar and his colleagues have studied 45 admitted patients and reported that 32 patients had 1/160 or higher levels by Wright test (71.11%) that is very similar to our results [11].

B. abortus antigen is used in the tube agglutination. It is reported those patients infected with B. melitensis have half the antibody titer than should be expected. It seems that brucellosis should be considered in those patients with clear serological evidence results. Therefore, looking for other laboratory results is essential and lower antibody titer can be considered as well. In a performed study, it is reported 58% of admitted brucellosis patients with

1/80 and lower titer of 2ME, brucella was isolated. It means low level of 2ME can be also significant as well. In our study, 29 patients had Wright negative results while 112 patients had no significant 2ME results [12].

Conclusion:

Those patients with no obvious serological results should be considered to have brucellosis because specific antibody does not rise as it is expected in all patients. Coombs test is recommended for those suspicious to have brucellosis and have negative Wright and 2ME tests.

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