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Original Article

Complete Blood Count, C-reactive Protein, and Erythrocyte Sedimentation Rate Changes in People with Brucellosis

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Abstract

Background: *Brucellosis is a major health and economic problem in many parts of the world, including the Middle East. Blood disorders such as anemia, leukopenia, and thrombocytopenia can be seen in brucellosis. However, laboratory findings of this disease are different. Therefore, this study aimed to investigate the changes in complete blood count (CBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) in people with brucellosis in Gonabad community health centers.*

Methods: *During the 4 years from May 2016 to May 2019, a prospective study was performed on 221 patients with brucellosis. According to the national guidelines for brucellosis, titers greater than 1/80 for Wright and more than 1/40 for 2-ME were considered positive cases of brucellosis. Using the checklist, information related to CBC, CRP, and ESR test parameters was collected.*

Results: *The results showed that out of 221 patients studied, 58.4% were male and 41.6% were female. The mean age was 44.9±19.8 years for men and 49.3±17.3 years for females. High ESR was seen in 43.4% and positive CRP in 59.7% of patients. Leukopenia in 8.6%, leukocytosis in 9%, neutropenia in 6.8%, neutrophilia in 9.5%, lymphopenia in 3.6%, lymphocytosis in 10%, anemia in 17.2% and thrombocytopenia in 9.5% of patients were seen.*

Conclusion: *Brucellosis in endemic areas should be considered in the differential diagnosis of patients presenting with thrombocytopenia. Also in high Wright titers, CRP is a more valuable indicator than ESR.*

Keywords*: Brucellosis, CBC, ESR, CRP*
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Introduction

Brucellosis is an irresistible illness caused by a gram-negative coccobacillus of the sort Brucella, with different clinical signs. More than half a million individuals are analyzed with brucellosis each year (1). This disease is observed all over the world, especially in the countries around the Mediterranean (Southern Europe, North, and East Africa), Middle East, India, and Central Asia(2).

Brucella melitensis and *Brucella abortus* are the most common disease-causing species, and most cases of disease in humans are caused by *Brucella melitensis* (3). Early and exact determination of this infection, in this manner, plays a vital part in controlling and eradicating brucellosis for making strides in well-bingeing. Different research facility tests, such as bacteriological, serological, and molecular methods, have been created to analyze brucellosis (4). Although brucellosis has been controlled in many developed countries, it remains an important health problem in developing countries(5).

Brucellosis is a multi-system disease and it has been reported to affect the digestive, cardiovascular, hematopoietic, nervous, skeletal, pulmonary, skin, and eye systems. This disease has many clinical variations and the symptoms can be differentiat-

ed from many infectious and non-infectious diseases (6).

Blood disorders such as leukopenia, anemia, thrombocytopenia, cytopenia,nd pancytopenia are seen in brucellosis, which can be mistakenly diagnosed as a hematological malignancy. The clinical and laboratory findings of brucellosis are non-specific and this should be considered in the differential diagnosis of patients with blood disorders(7).

On the other hand, the laboratory findings of this disease are different in various studies and populations. Therefore, this study is conducted to investigate the changes in complete blood count (CBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) in people with brucellosis who refer to health and community health centers in Gonabad city.

Materials and method

Blood sampling and processing

During 4 years from May 2016 to May 2019, a retrospective study of 221 patients with brucellosis was investigated. The sampling method was a census and checklist of information related to age, gender, test date, blood parameters such as CBC test parameters (WBC and their differential count, RBC count, hemoglobin measurement, hematocrit, and platelet count), CRP, ESR and Brucellosis diagnostic serological tests including Wright test and 2-mercaptoethanol (2-ME) were performed for each patient.

Sample size

Using the following formula and with the help of Nasaji et al.'s study (8), also considering the confidence level of 95%, the sample size in this study was calculated to be 174 people, and taking into account 15% possible dropout, the final sample size was determined to be 200 people.

$$n = \frac{z_{1-\frac{\alpha}{2}}^{2} * p * (1-p)}{d^2} = 174$$

where: n = the required sample size; P = estimated prevalence = 0.5; z = level of confidence as 1.96 and d = desired precision level = 0.05.

Diagnosis method

The results of serological diagnostic tests for Brucellosis including Wright and 2-ME were extracted and according to the national guidelines for Brucellosis, titers greater than 1/80 for Wright and greater than 1/40 for 2-ME were considered positive cases of Brucellosis. By using the checklist, relevant information was collected, and based on normal ranges, low, normal and high values were determined for each variable and analyzed.

Statistical analysis

After collecting and entering data into SPSS software version 16, central (mean) and dispersion (standard deviation) indicators were used to describe quantitative variables and frequency and frequency percentage were used to describe qualitative variables. In the analytical analysis, the chi-square test, Kruskal-Wallis's test, and Fisher test were used to investigate the relationship between qualitative variables. A logistic regression test was also used to examine some variables. $P<0.05$ was considered as significance level.

Results

In this study, out of 221 patients with brucellosis, 129 (58.4%) were male and 92 (41.6%) were female, and the average age of the patients was 46.7–18.9 years. There was high ESR in 43.4% and normal ESR in 56.6% of the studied subjects. There was negative CRP in 40.3% and positive CRP in 59.7% of patients with brucellosis. The frequency results of the Wright and 2ME tests of the studied patients are listed in Table 1.

Table 1: Frequency of the Wright and 2ME test.

Test	Titter	Frequency (%)
Wright	1.80	63(28.5)
	1.160	44(19.9)
	1.320	40(18.1)
	1.640	37(16.7)
	1.1280	26(11.8)
	1.2560 and higher	11(5.1)
2ME	1.40	82(37.1)
	1.80	45(20.4)
	1.160	40(18.1)
	1.320	29(13.1)
	1.640	20(9)
	1.1280	3(1.4)
	1.2560 and higher	2(1)

The CBC examination of the subjects showed leukopenia in 8.6%, leukocytosis in 9%, neutropenia in 6.8%, neutrophilia in 9.5%, lymphopenia in 3.6%, lymphocytosis in 10%, anemia in 2.2%, polycythemia in 0.5%, thrombocytopenia in 9.5%, and thrombocyto-

sis in 0.9% of the studied subjects.

In this study, no one had pancytopenia, while 11 patients (5%) had cytopenia, of which 8 patients had leukopenia with thrombocytopenia, 2 patients had leukopenia with anemia, and only one patient had thrombocytopenia with anemia.

In this study, there was no significant relationship between Wright titer and age (P = 0.431) (Table 2), incidence of leukopenia (P = 0.474), neutropenia (P = 0.475), lymphopenia (P = 0.352), anemia (P = 0.705), or thrombocytopenia (p 0.001) (Table 3). There was a significant correlation between the Wright titer and gender (P = 0.004), so the highest frequency was related to the group of females with a Wright titer rate of 1/80 (Table 4).

Table 2: Correlation between the Wright test and age.

Wright titer	**± mean) age (standard deviation**	**p-value**
1.80	48.2 ± 16.7	
1.160	50.2 ±20.2	
1.320	46.3 ± 20.1	0.431
1.640	43.8 ± 20.1	
1.1280	44.6 ± 15.9	
1.2560 and higher	40.8 ± 22.9	
Total	46.7 ±18.9	

Table 3: Frequency distribution of leukopenia, neutropenia, lymphopenia, anemia and thrombocytopenia and investigating the relationship between them and Wright titer

Variable	**Wright titer frequency (%)**							**p-value**
		1.80	1.160	1.320	1.640	1.1280	1.2560 And higher	
Leukopenia	Yes	7 (3.2)	5 (2.3)	2 (0.9)	1 (0.5)	2 (0.9)	2 (0.9)	0.474
	No	) 56 (25.3	39 (17.6)	38 (17.2)	36 (16.3)	24 (10.9)	9 (4.1)	
Neutropenia	Yes	3	3	5	3	0	1	0.475
	No	(1.4) 60 (27.1)	(1.4) 41 (18.6)	(2.3) 35 (15.8)	(1.4) 34 (15.4)	(0) 26 (11.8)	(0.5) 10 (4.5)	
	Yes	2	4	1	1	0	0	
Lymphopenia	No	(0.9) 61 (27.6)	(1.8) 40 (18.1)	(0.5) 39 (17.6)	(0.5) 36 (16.3)	(0) 26 (11.8)	(0) 11 (5)	0.352
	Yes	7	8	9	8	4	2	
Anemia	No	(3.2) 56 (25.3)	(3.6) 36 (16.3)	(4.1) 31 (14)	(3.6) 29 (13.1)	(1.8) 2 (10)	(0.9) 9 (4.1)	0.705
	Yes	3	3	2	3	9	1	
Thrombocytopenia	No	(1.4) 60 (27.1)	(1.4) 41 (18.6)	(0.9) 38 (17.2)	(1.4) 34 (15.4)	(4.1) 17 (7.7)	(0.5) 10 (4.5)	P<0.001

Table 4: Correlation between the Wright test and gender

Wright titer	Gender		p-value
	female (%)	male (%)	
1.80	35(15.8)	28(12.7)	
1.160	22(10)	22(10)	
1.320	12(5.4)	28(12.7)	0.004
1.640	16(7.2)	21(9.5)	
1.1280	6(2.7)	20(9)	
1.2560and higher	1(0.5)	10(4.5)	
Total	92(41.6)	129(58.4)	221(100)

The results of this study showed that there is a significant relationship between high ESR titers ($P < 0.001$) (Table 5) and positive CRP ($P < 0.001$) (Table 6), that the highest frequency is related to the normal ESR group with a Wright titer rate of 1/80, and the highest frequency is related to negative CRP with a Wright titer rate of 1/80, and patients with a Wright titer rate of 1/1280 had the highest frequency of thrombocytopenia.

Table 5: Correlation between high ESR with Wright titer

Wright titer	Coefficient	SE	OR	Confidence interwall For OR	p-value
1.80	Reference level				
1.160	0.79	0.43	2.20	0.94–5.15	0.068
1.320	1.76	0.44	5.83	2.43–13.69	p<0.001
1.640	1.52	0.44	4.59	1.90–11.08	0.001
1.1280	1.25	0.49	3.50	1.32–9.24	0.011
1.2560 And higher	1.43	0.67	4.20	1.11–15.83	0.034

Table 6: Correlation between positive CRP with Wright titer

Wright titer	Coefficient	SE	OR	Confidence interwall For OR	p-value
1.80	Reference level				
1.160	1.25	0.41	3.50	1.56–7.84	0.002
1.320	1.54	0.43	4.16	1.98–10.97	p<0.001
1.640	1.42	0.44	4.66	1.75–9.89	0.001
1.1280	2.12	0.56	8.40	2.77–25.41	P<0.001
1.2560 And higher	2.19	0.82	9	1.78–45.44	0.008

The chance of a positive CRP index in people with a titer of 1/160 is about 3.5 times that of patients with a titer level of 1/80, and this chance ratio for people with a titer level of 1/2560 reaches 9 and its maximum value. The chance of having a high ESR in people with a titer of 1/160 is about 2.20 times that of patients with a titer level of 1/80, and this chance ratio reaches its maximum (5.83) in patients with a titer level of 1/320. And further, with the increase in Wright titer level, the chance ratio of people decreases, so that at the titer level of 1/2560, the chance of having a high ESR in a person is 4.20 times that of a person at the titer level of 1/80.

Discussion

The present study was conducted to determine the changes of CBC, CRP and ESR in patients with brucellosis who were referred to health and community health centers in Gonabad city, and in this regard, the changes of these blood parameters were investigated in 221 patients with brucellosis. In this study, the number of positive cases was higher in men, and the higher prevalence of brucellosis in men can be attributed to work-related infections and stronger humoral and cellular immune systems in female.

The results of the present study showed that high ESR is present in 43.4% of affected patients. This amount has been stated in the studies conducted in Iran (29.5%)(9), India (80%) (10), Iran (38%) (11), Iran (44.5%) (12), Iran (45.3%) (13).

Regarding CRP variable, in our study 59.7% of people with brucellosis had positive CRP. This amount in different studies conducted in Iran were (34%) (9), (63%) (11), (45%) (12), (69.2%) (13).

In our study, comparing the frequency of high ESR and positive CRP in patients with brucellosis showed that positive CRP cases (59.7%) are more than high ESR cases (43.4%). ESR and CRP are two tests to check the presence of inflammation and hidden infections in the body, but CRP has higher sensitivity and specificity than ESR, which has been shown in our study.

The results of ESR test in brucellosis have many challenges because the increase of IgM pentamer, or the excessive increase of IgG titer increases the plasma viscosity and causes no increase in ESR. In brucellosis, depending on the stage of the disease, we may have high or normal ESR, which is affected by plasma viscosity. In such cases, CRP is better because it is not affected by plasma viscosity. Therefore, CRP results are more valuable than ESR. According to our investigation, CRP positive level increased with Wright titer increasing, but high ESR cases did not increase with Wright titer increasing.

Our study showed that leukopenia exists in 8.6% of patients with brucellosis. While in the studies conducted in Iran (31.8%) (9), Iran (8.5%) (14), India (14.7%) (10), Iran (33%) (11), Iran (9%) (12), Turkey (85.5 %) (15), Iran (23.1%) (13), Israel (28%) (16) were seen.

In this study, leukocytosis was seen in 9% of patients with brucellosis, and neutropenia was found in 6.8% and neutrophilia in 9.5% of patients under study. Also, our study showed that there is lymphopenia in 3.6% and lymphocytosis in 10% of the patients under study.

In the present study, anemia is seen in 17.2% of patients with brucellosis. This amount in different studies in Iran (56.8%) (9), Iran (42.6%) (14), India (57.3%) (10), Iran (53%) (11), Iran (19%) (12), Turkey (4.3) (15), Iran (52.1%) (13), India (21.4%)(17) and Israel (13%) (16).

According to our results, thrombocytopenia was present in 9.5% of patients with brucellosis. This amount in different studies in Iran (9.1%) (9), Iran (12.5%) (14), India (33.82%) (10), Iran (12%) (11), Iran (7.4%) (12), Turkey (15.0%) (15), Iran (15.4%) (13), India (18.2%) (17) and Israel (14%) (16).

In our study, none of the patients with brucellosis had pancytopenia, which is similar to the study of Fanni et al.'s (11) as well as the study of Balın et al.'s (3). While in Behnaz et al.'s study (14), pancytopenia was found in 1.5%, in Hoseini et al.'s study in 1.6% (12), in Kaya et al.'s study in 5.7% (17). And in the study of Justman et al., it was present in 2% of affected patients (16).

In our study, bicytopenia is present in 5% of people with brucellosis, which is 4.8% in Hoseini et al.'s study (12) and 4.3% in Balın et al.'s study (3).

In our study, there was a correlation between the titer and sex, so that most female had a titer of 1.80, but in the study of Hoseini et al. (12), there was no correlation. In explaining this case, it can be said that female have a stronger humoral and cellular immune system than men, and as our study shows, female have lower titers of Wright in case of brucellosis.

In the present study, there was no relationship between Wright titer and age, while in the study of Hoseini et al. (12), there was a significant relationship between Wright titer and age over 45 years.

In our study, a statistically significant correlation was observed between Wright titer and ESR, so most people who had normal ESR had a 1/80 Wright titer, but in the study of Hoseini et al. (12), no correlation between two- titer was observed. At low Wright titer levels, the antibody titer is low, the zeta potential of the RBC level is less neutralized and ESR has not increased due to its low sensitivity. As the Wright

titer increases, the ESR also increases, but in cases where the titer is too high, the ESR does not increase due to the increase in plasma viscosity(18, 19).

In the current study, a statistically significant correlation was observed between wright titer and CRP, so most people who had negative CRP had a 1/80 Wright titer, and also in the study of Hoseini et al. (12), the correlation between Wright titer and positive CRP was significant.

The results of this study regarding the relationship between the chance of positive cases of ESR and CRP tests with the antibody titer showed that the graph of ESR changes with the Wright titer is a bell-shaped graph, which is the highest value in the titer of 1/320 and in values higher than this titer, the rate of ESR positivity decreases, but in the case of CRP, with the increase in the Wright titer, the chance of positive cases of this test increases, and this finding shows the effect of the increase in plasma viscosity on the results of the ESR test. In higher titers, due to the increase in the number of antibodies in the plasma, the viscosity increases, and on the other hand, the RBC sedimentation rate decreases, but in the lower titers, due to the absence of plasma hyper-viscosity and the neutralization of the zeta potential. The surface of the RBC forms roulo is more and then the rate of sedimentation of the red blood cells increases (20).

In addition to the diagnostic value of Brucellosis, the two tests ESR and CRP have the value of predicting specific complications and organ involvement. According to various studies (21, 22), two tests, ESR and CRP, especially ESR can be used as an inflammatory biomarker in the prognosis of complications and organ involvement. Therefore, the results of this test are important for doctors in brucellosis and not only cover the issue of diagnosis, but also can be useful for doctors in terms of prognosis. Furthermore, the accuracy of the results of this test should be investigated and paid attention to according to the effectiveness of this test in higher titers. However, it is suggested that the investigation of plasma viscosity should also be taken into consideration in the diagnosis and prognosis program of brucellosis disease.

In this study, a statistically significant relationship was observed between the Wright titer and the incidence of thrombocytopenia, so that the lower the titer, the lower the incidence of thrombocytopenia, and conversely, the higher the Wright titer, the higher the incidence of thrombocytopenia; While in the study of Hoseini et al. (12), there was no connection between the two-titer observed.

In our study, there was no statistically significant relationship between leukopenia, neutropenia, lymphopenia, and anemia with Wright titer, but it was significant for the correlation between Wright titer and thrombocytopenia. Thrombocytopenia, like anemia, is a sign of the existence of another disease, which has many differential diagnoses in its diagnostic approach; Therefore, according to the prevalence of brucellosis in the region, it is possible to consider brucellosis as one of the priorities of differential diagnoses for the diagnostic approach to thrombocytopenia in the case of patients presenting with thrombocytopenia, rather than spending time and money on diagnostic procedures. It should be avoided due to the prevalence of thrombocytopenia in these patients.

Conclusion

The results of this study showed that according to the prevalence of brucellosis in the study area, changes in CBC test, especially thrombocytopenia, can make brucellosis more colorful in the differential diagnosis of thrombocytopenia. Also, the results of two ESR and CRP tests showed that the CRP test has more diagnostic value than ESR due to its higher sensitivity and specificity, and in high Wright titers, the results of the ESR test should be interpreted with caution and the results of the CRP test as well as plasma viscosity should be taken into account.

Data Availability

Data are available on request from the authors.

Ethical Approval

This study was approved by the Research Ethics Committee of Gonabad University of Medical Sciences; Gonabad, Iran, with code number IR.GMU.REC.1398.154.

Conflict of interest

The authors have no conflict of interest to declare.

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