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Clinical and Hematological changes associated with brucellosis in Missan Province

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Abstract

Objective: Brucellosis is still endemic in Iraq and the main source of public health risk; this study was performed to investigate the clinical signs and symptoms of Brucellosis and determine the hematological changes in the patients infected with *Brucella* which may assist in the rapid diagnosis of the disease.

Subjects and Methods: The study was performed in a period extending from (the first of January -2021to to the Third of June-2021). The data and blood samples were collected from (184) outpatients from a big hospital (Al-Sadder) in Missan Province infected with brucellosis aged between (10 - 65) years. the patients were diagnosed with brucellosis according to case history and clinical laboratory examination. The blood sample from each patient was divided into two test tubes, the first (2ml) for serological examination through separated the serum from the blood components and stored at (-18 °C) until analysis by using the Rose Bengal test. The hematological assessment was performed in the laboratory for each sample of blood (3 ml with EDTA) obtained from the patients infected with brucellosis. Complete blood counts were shown from the collected blood samples by automatic methods (System X kx-21n automated hematology analyzer; JAPAN CARE CO., LTD) including Hemoglobin (Hb), Leukocytes or white blood cells (WBCs), Platelets, Lymphocytes, and Neutrophiles. *Results:* The disease was diagnosed in the patients by using the Rose Bengal test. the results observed a strong positive reaction for the patients infected with brucellosis**,** the majority and more than half of brucellosis cases were recorded in females (58.3%), and males (41.7%) of all cases. High incidence of brucellosis in the ages between (41-50) years (33.6%) cases, followed by the ages between (31 -40) years (31.1%) cases, while the lowest percentage infected with the disease was reported in ages between (10 -20) years in (8.1%) cases**.** The most common symptoms observed were fatigue (85.3%), fever (77.7%), arthritis (73.3%), sweats (69%), and headache (64.1%). The hematological analysis for the blood samples observed significant changes in (*P*<0.05) in leukocytes, leukocytosis (23.8%), leucopenia (12.2%), Anemia reported in (42.7%), Platelets also reported in abnormal value, thrombocytosis (15.1%), thrombocytopenia (11.1%) of all cases of brucellosis. lymphocytosis (32.7%), lymphocytopenia (15.5%), while the neutrophils reported in abnormal ranges , neutrophilia (15.5%),neutropenia (11.6%) .*Conclusions:* the brucellosis prevalence as a zoonotic disease in rural areas in Missan and direct contact and handling of livestock make the females highly exposed to the risk of infection. The blood picture reveals hemolytic anemia, leukopenia, lymphocytosis, lymphocytopenia, and Neutrophils with neutropenia particularly when the disease is epidemiologically suspected.

Recommendations: Some of the population tend to rear animals inside the houses, which demanded specific authorities to take care control of the disease, especially in the animals that consider the main source of the disease.

Introduction

Brucellosis is a bacterial zoonosis transmitted directly or indirectly to humans from infected animals. It constitutes a major health problem in many parts of the world, particularly in the Mediterranean and Middle East (Liae *et al*, 2014), this disease became emerging disease Since the causative agent *Brucella melitensis* was discovered in 1887 by David Bruce (Corbel,2006).

Brucella infection is transmitted to humans by contact with fluids from infected animals (sheep, cattle, goats, pigs, or other animals) or derived food products such as unpasteurized milk and cheese. Brucellosis has high morbidity both for humans and animals; it is an important cause of economic loss and public health problems in many developing countries (Alzubaidy,2008; Guler *et al*,2014).

Dermott *et al* (2013) reported that humans contract the disease by ingesting contaminated food, such as unpasteurized milk products, but can also acquire it through inoculation via direct contact with infected animals or animal parts and occasionally through occupational exposure in microbiology laboratories.

In Iraq, the brucella hit humans and animals together in all governorates, since its first time discovered until know days, in human many cases have been recorded in different governorates, from 2001 up to 2003 recorded 108 incidences in Baquba city (Al-Dilamy,2010), while in 2004 Khuder (2006) was recorded 166 infection case in Salahddin province where notice the ratio of female infection reached to 96.6%.

The *Brucella* may enter the body through the digestive tract, lungs or mucosal layers, and intact skin. Then it may spread through blood and the lymphatic system to any other organ where it infects the tissues and causes localized infection (Lapaque *et al.,* 2005). The organism can escape phagocytic killing by inhibiting the phagosome-lysosome fusion and reproducing inside macrophages (Alkahtani *et al,* 2020).

After a variable incubation period ranging from less than one week to several months, non-specific systemic symptoms such as fever, headache, malaise, night sweats, and arthralgia follow, resembling a flu-like disease. During the early stage of the disease, patients are frequently bacteremic that have a continuous pattern, making circulating *Brucella* easily detectable by blood culture. Once in the bloodstream, the organism is seeded to multiple organs/systems, especially those rich in reticuloendothelial tissue, such as the liver, spleen, skeletal and hematopoietic system (Poester *et al*, 2013).

Clinical manifestations of brucellosis in adults, where clinical presentations of human brucellosis range from non-specific and constitutional symptoms, like prolonged fever, anorexia, or fatigue to local organ involvement, such as arthritis and neurobrucellosis. However, in children being unfamiliar with the disease may be a delay in diagnosis (Feiz *et al*,1978; Donev *et al*,2010).

The diagnosis of brucellosis requires the isolation of Brucella from blood or body tissues or the combination of suggestive clinical presentation and positive serology. The Rose Bengal test, complement fixation test, standard agglutination test), competitive enzyme-linked immunosorbent assay, as well as real-time PCR, is used for diagnosis (Guler *et al*, 2014; Nthiwa *et al*,2019).

Akdeniz *et al* (1998) referred to hematological disturbances in brucellosis are common and including anemia, leukopenia and thrombocytopenia can be encountered during the disease, which can be misdiagnosed as a hematologic malignancy. Also founds in Turkey 233 patients showed leukopenia in 21%, anemia in 44%, thrombocytopenia in 26%, and pancytopenia in 8%. Another study of hematological changes during the active course of brucellosis showed that leucopenia occurs in 45%, anemia in 74%, thrombocytopenia in 39.5%, and pancytopenia in 21% (Yumuk and O'Callaghan, 2012).

The aim of this study was performed to investigate the clinical signs and symptoms of

Brucellosis and to determine the hematological changes in the patients infected with *Brucella* which may assist in rapid diagnosis of the disease.

Materials and methods

The study was performed in the period extending from (the first of January -2021 to the Third of June-2021). The data and blood samples were collected from (184) outpatients from a big hospital (Al-Sadder) in Missan Province infected with brucellosis aged between (10 - 65) years, the patients were diagnosed with brucellosis according to case history and clinical laboratory examination.

The venous blood samples(5 ml) were taken from the patients for serological and hematological examinations, and then the blood sample from each patient was divided into two test tubes, the first (2ml)for serological examination through separated the serum from the blood components by using centrifugation (3000rpm)for (5 minutes)after that the serum stored at   (-18 °C) until analysis by using Rose Bengal test according to the method that described by Amusawi (2017) as following:

- Rose Bengal test (rapid test developed for detection of Brucella antibodies in human and animal sera through agglutination that occurs between *Brucella abortus* S-99 and antibodies type *IgM and IgG* in patients' serum.
- Rose Bengal Kit that was used in this study was obtained from Omega Diagnostic Company (Scotland)and contains *Brucella abortus* S-99 killed by heat and treated with phenol 5% and Tri –HCL in PH 3.62 and stained with Rose Bengal dye.
- The test was done by dispensing (50 ul) of each serum by micropipette to be tested on an enamel plate. The same amount of Rose Bengal antigen was added to each serum and mixed and shacked by hand for 4 minutes, and the test result was then read. Agglutination appeared as a weakly positive, positive, strongly positive, or very strong positive.

The hematological assessment was performed in the laboratory for each sample of blood (3 ml with EDTA) obtained from (184) patients with infected with brucellosis. A complete blood picture was shown from the collected blood samples by automatic methods (System X kx-21n automated hematology analyzer; JAPAN CARE CO., LTD) including Hemoglobin (Hb), Leukocytes or white blood cells (WBCs), Platelets, Lymphocytes, and Neutrophiles.

Statistical analysis.

Statistical analyses were made with a one-way analysis of variance (ANOVA) using SPSS 17. The criterion for statistical significance was ($P<0.05$).

The results and Discussion

The results of this study observed the information about the disease from the aspect of age, gender, and the commonest clinical signs and symptoms. The disease was diagnosed in the patients by using the Rose Bengal test. the results observed a strong positive reaction for the patients infected with brucellosis may be due to high titration of antibodies and strong stimulation of B- lymphocytes.

Al-Thawani (2000) was refereed that the Rose Bengal test used to detect brucellosis even with low titration of antibodies and we can determine the early stages of the disease due to the presence of antibodies type IgM in the acute stage and IgG in chronic disease.

Al-Mousawi (2017) found strong and clear agglutination in the sera for some cases of children and adolescents diagnosed with brucellosis.

The result observed that the majority and more than half of brucellosis cases were females (58.3%) and the males in this study (41.7%) of all cases, Fig (1).

WHO (2006) recorded that the higher incidence of brucellosis among human females than males might be due to the females in rural area's direct contact and handling of livestock, which make the females highly exposed to the risk of infection with the animals, consumption of raw milk and milk products. Moreover, risky practices in rural areas such as skinning of stillborn lambs and kids, as well

as crushing the umbilical cord of newborn lambs and kids with teeth can also be contributing factors. Tofah (2008) found the females more infected than the male in a study performed on (300) patients in Al-Kut city by using the Rose Bengal test. But these results not in agreement with a study was performed by Cekanac *et al* (2010) in Serbia in years between (1980- 2008) on 1521 persons infected with brucellosis and found the males more infected than the females because the males were in direct contact with domestic animals than the female during lactation and parturition. Consumption of dairy products such as raw milk, soft cheese, butter, and ice cream consider the most important source of infection, also raw vegetables and water are contaminated through excreta, and sometimes

consumption of undercooked animal meat though the bacterial load is very low. Man becomes infected by handling infected tissues of animals, by close contact with other infected materials where Brucella enter through skin abrasions, trans-conjunctiva, and air born transmission are also indicated. Therefore, brucellosis is an occupational disease of the stockyard, slaughterhouse workers, bluchers, and veterinarians (Kiros *et al,*2016; Ali *et al,*2018).

The results observed a high incidence of brucellosis in the ages between (41-50) years in percentage (33.6%) cases, followed by the ages between (31 -40) years in percentage (31.1%)cases, while the lowest percentage infected with the disease reported in ages between (10 -20)years in (8.1%) cases, Fig(2).

		Males	Females
■ No		35	49
■ %		41.66	58.33
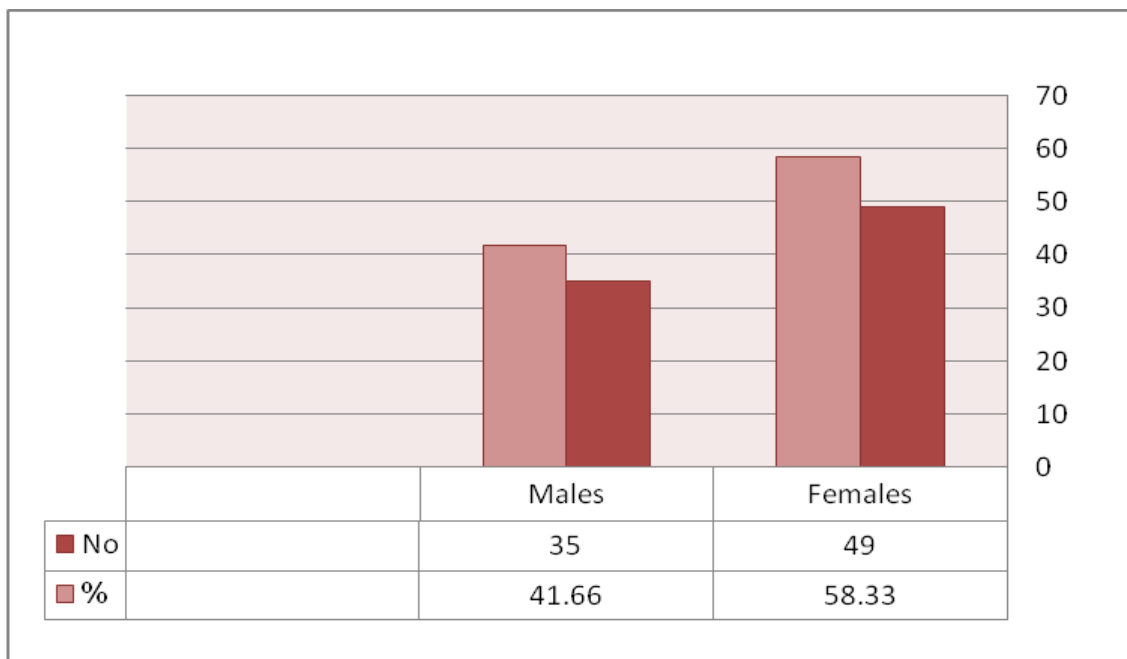
Finger (1) The total number of females and males infected with Brucellosis
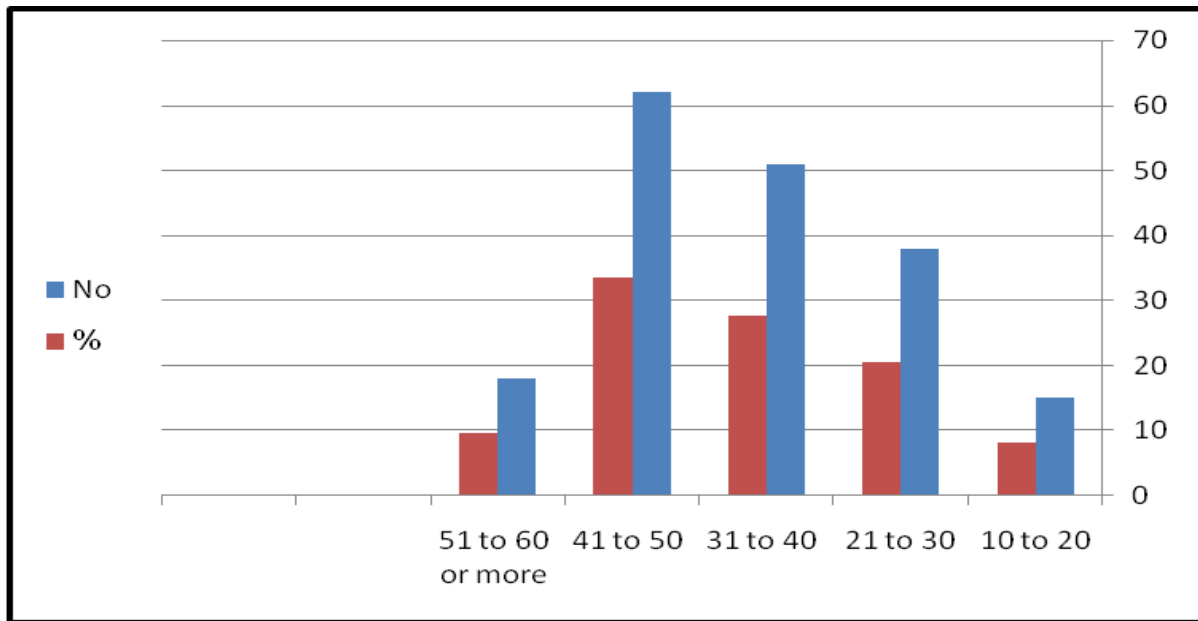
Fig (2) Show the distribution of Brucellosis among the patients according to the different ages.

Cekanac *et al* (2010) were reefer the most common ages infected with brucellosis in between (30-49) years. Also, Germany found a relationship between age and infection with brucellosis, therefore the disease considers an occupational disease (Dahouk *et al*,2007). Salamah *et al* (2008) found a high distribution of brucellosis in humans and form (8%) in Egypt due to direct contact with infected animals and half of the patients were from farmer families and rural areas. But the results of this study are not in agreement with another study performed in California in the USA which found an increase in brucellosis infection in ages (55-64) years due to consumption of unpasteurized dairy products or contact with domestic (Ajello and Mays,1998**;** Ali *et al*,2021).

A study performed in Iran by Aghali *et al* (2015) found that younger boys in adolescents ages are more susceptible to being infected with brucellosis, especially in rural areas and occupational contact with animals was unavoidable; so, older children

might be at increased risk of contact with the infected animals. Al –Hussain, and Thaer (2012) detected many factors affect the infection of brucelloses such as climatic conditions, geography, species, sex, and age and most cases of brucellosis were reported in Iraqi cities of middle and south Iraq (Baghdad, Salah-Eldin, Wasit, Babil, Thiqar, Missan &Basra).

Al-Dewan (2007) referred that brucella found high prevalence in developing countries and consider a big problem in public health and the percentage may be reached (80%) in dairy products fields.

The frequencies of clinical signs and symptoms in the patients infected with brucellosis are represented in fig (3), where the most common symptoms observed are fatigue (85.3%), fever (77.7%), arthritis (73.3%), sweats (69%), headache (64.1%), while the lowest clinical signs and symptoms were weight loss (29.8%), back pain (34.2%), anemia (38.6).
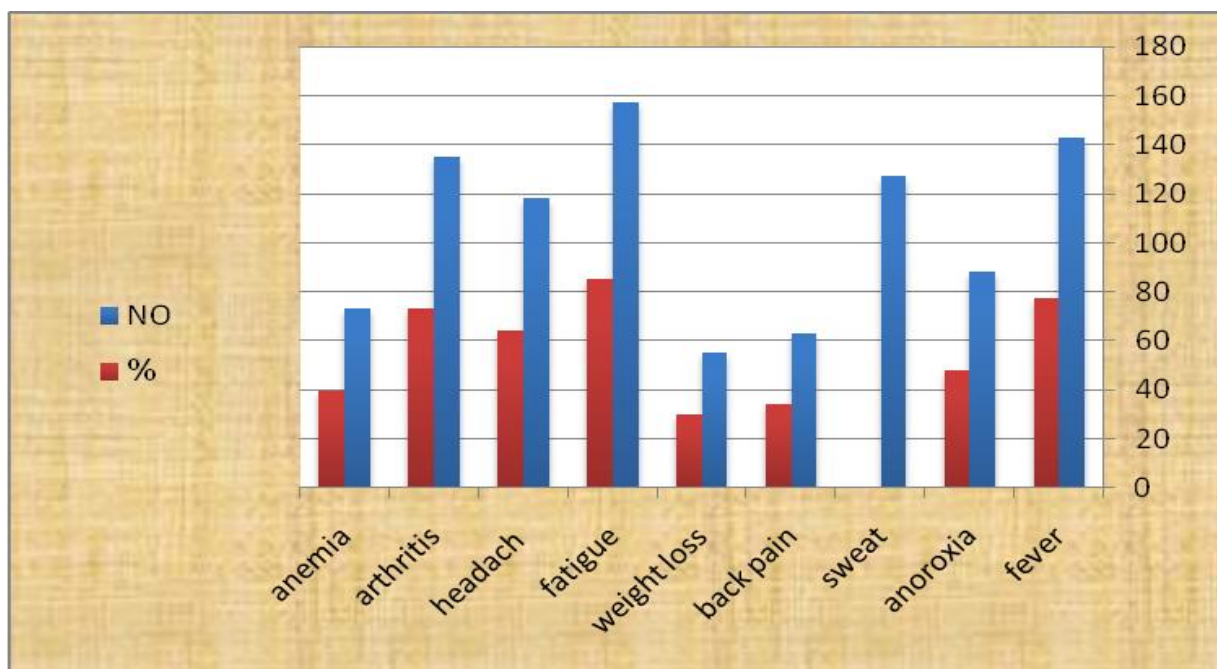
Figure (3) Show clinical signs and symptoms in the patients infected with brucellosis.

Corbel (2006) said the incubation period of the disease is between 2-3weeks and the acute phase may be found in most patients infected with brucellosis with nonspecific symptoms including fever, sweating at night, headache, loss of appetite and arthritis, and the fever differ during long times there for the disease sometimes called undulating fever.

Zamani *et al* (2011) reported that the chronic stage of the disease may not be accompanied by symptoms, sometimes with undulating fever, and death may occur in 2% of untreated cases due to pericarditis. Al-Nassir (2014) found that most of the signs and symptoms of brucellosis are accompanied with increase fever, sweating during the night times, chills, and arthritis especially in the Sacroiliac joint, knee, and ankle joint. Ramezani (2008) reported that the complications of brucellosis are a major medical problem in countries and are still endemic, these complications include osteoarticular, gastrointestinal, hematological, genitourinary, cardiovascular, respiratory, and central nervous system involvement.

The hematological analysis for the blood samples obtained from the patients infected with Brucellosis observed significant changes in leukocytes, where findings of leukocytosis (23.8%), leukopenia (12.2%), and the normal ranges of leukocytes reported in (63.8%). Hemoglobin concentrations also observed abnormal changes in patients with brucellosis, Anemia was reported in (42.7%), and the normal ranges of hemoglobin were found in (57.2%).

Platelets were also reported in abnormal values in this study, thrombocytosis (15.1%), and thrombocytopenia (11.1%) in all cases of brucellosis. Lymphocytes were also found in abnormal values in patients with brucellosis, where lymphocytosis (32.7%), lymphocytopenia (15.5%), and the normal range of the lymphocytes were recorded at (51.6%). Neutrophils were reported in abnormal ranges in patients with brucellosis, where neutrophilia (15.5%), neutropenia (11.6%), and the normal percentage of neutrophils were found in (72.7%), Tab (1).

Table-1: Show Hematological changes in patients infected with Brucellosis (N: *180).

Parameters	Hematological finding	N O	%
Leukocytes (mm3)	Normal (4000-10000)	115	63.8%
	Leukocytosis (>10000)	43	23.8%
	Leucopenia (	22	12.2%
Hemoglobin Hb(g/dl)	Normal (13-18)	103	57.2%
	Anemia (12)	77	42.7%
Plate lets (Plt)	Normal (130-400)	133	73.8%
	Thrombocytosis ((>400.000)	27	15%
	Thrombocytopenia (>130.000)	20	11.1%
Lymphocytes(1u/L)	Normal (1000-4800)	93	51.6%
	Lymphocytosis (>5000)	59	32.7%
	Lymphocytopenia (4000)	28	15.5%
Neutrophiles (%)	Normal (55-70%)	131	72.7%
	Neutrophilia (Neutrocytosis) (>70%)	28	15.5%
	Neutropenia (50%)	21	11.6%

*Only 180 patients agreed to the hematological analysis.

Figure (4) observed the changes in leukocytes in patients with brucellosis, leukocytosis (23.8%), leukopenia (12.2%), and the normal ranges of leukocytes reported in (63.8%).

Figure (5) show abnormal changes in hemoglobin concentrations in patients with brucellosis, and anemia was reported in (42. %) of all cases, while the normal range of hemoglobin was found in (57.2%).

Figure (6) shows changes in the platelets, where reported thrombocytosis (15.1%), thrombocytopenia (11.1%), and the normal range of platelets found in (73.8%).

Lymphocytes in this study were found in abnormal ranges in some cases with brucellosis, lymphocytosis (32.7), and lymphocytopenia (15.5%), while the normal ranges of lymphocytes were found in (51.6%), fig (7).

Neutrophils were found in abnormal values in some cases of the patients, where the neutrophilia were found in (15.5%), neutropenia (11.6%), while the normal range of the neutrophils was found in (72.7%), fig (8).
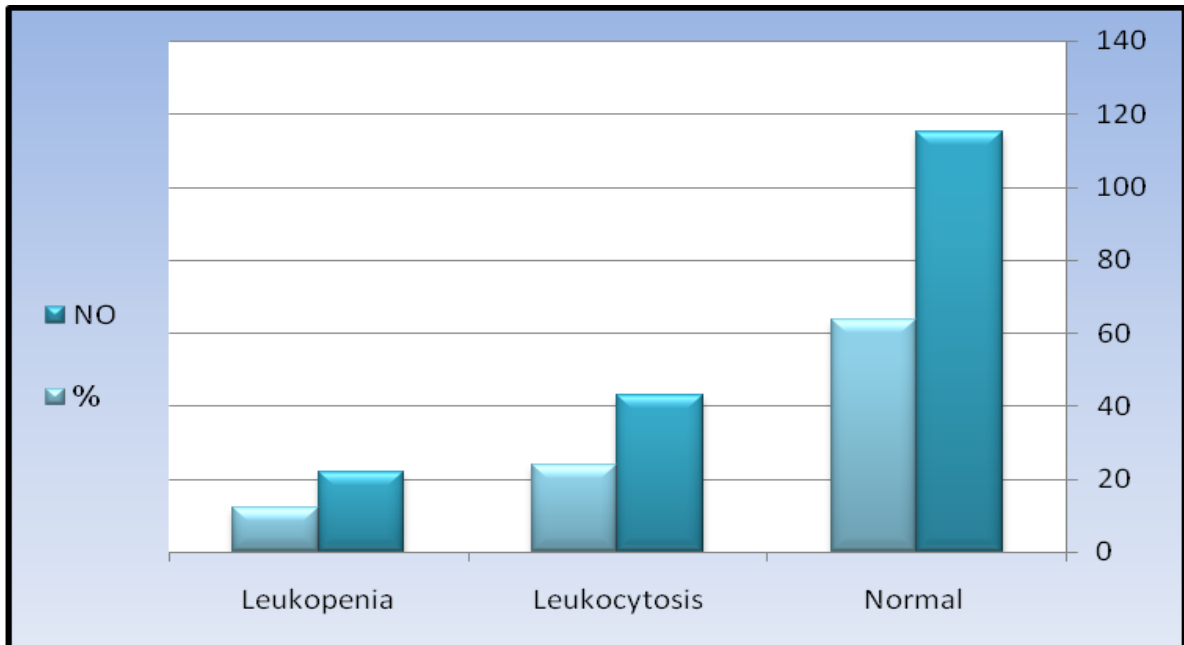
Figure (4) Show the changes in leukocytes (WBCs) in patients infected with Brucellosis as compared to normal values
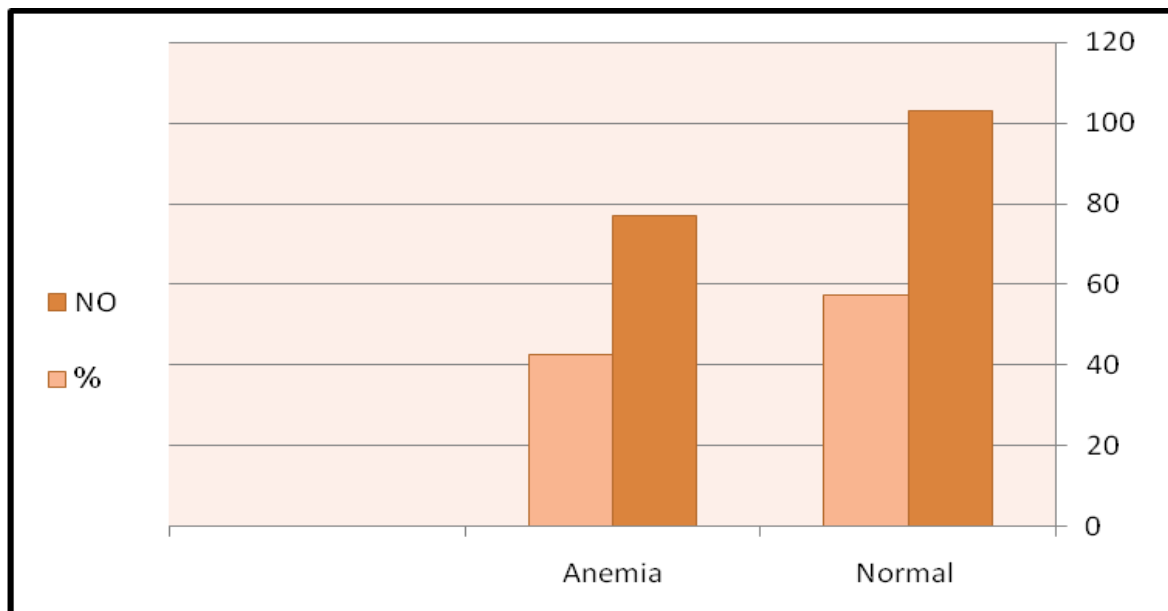

Figure (5) Show the changes in Hemoglobin (Hb) in patients infected with Brucellosis as compared to normal values
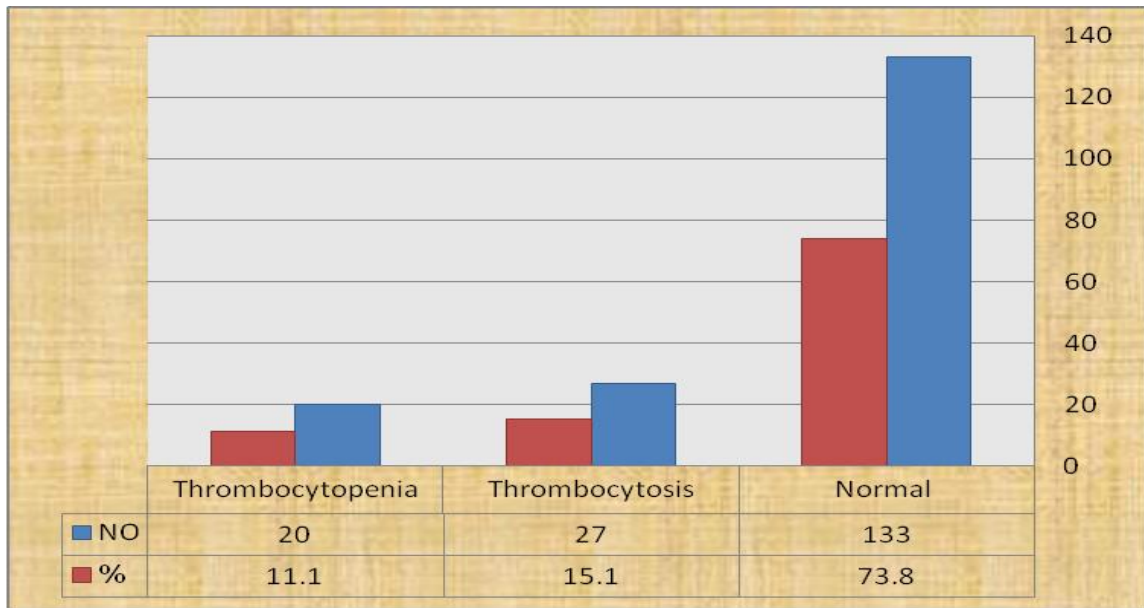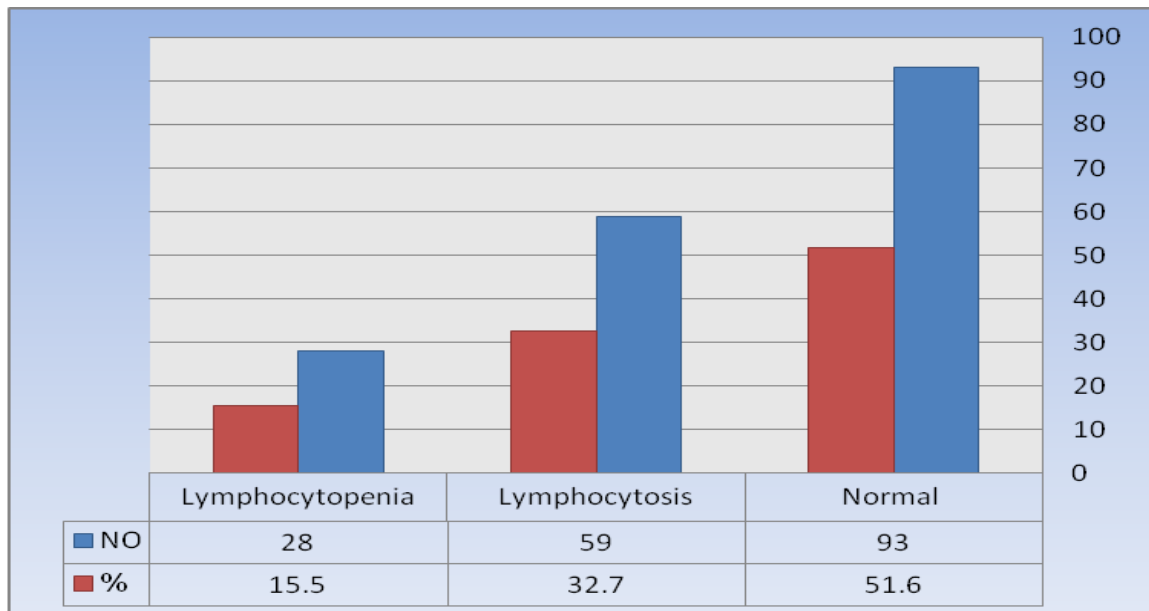
	Thrombocytopenia	Thrombocytosis	Normal
NO	20	27	133
%	11.1	15.1	73.8

Figure (6) Show the changes in Platelets (Plt) in patients infected with Brucellosis as compared to normal values

	Lymphocytopenia	Lymphocytosis	Normal
NO	28	59	93
%	15.5	32.7	51.6

Figure (7) Show the changes in (Lymphocytes) in patients infected with Brucellosis as compared With normal values.

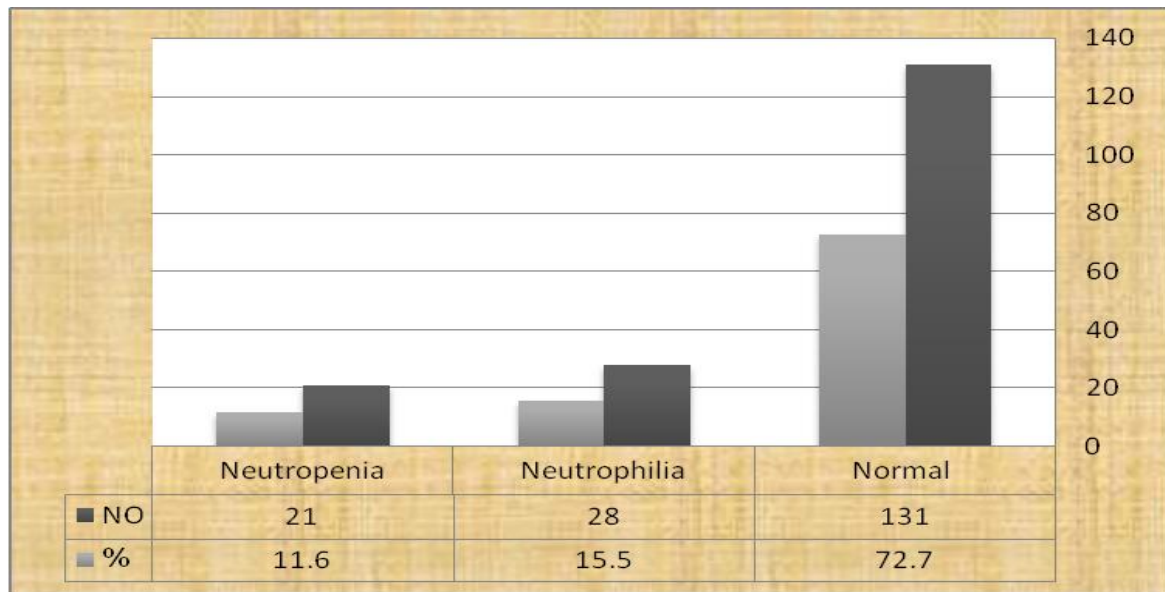|	Neutropenia	Neutrophilia	Normal
■ NO	21	28	131
■ %	11.6	15.5	72.7

Figure (8) Show the changes in (Neutrophiles) in patients infected with Brucellosis as compared To normal values

Crosby *et al* (1984) detected leucopenia in (45 %), anemia (74%), thrombocytopenia (39%), and pancytopenia in (21%) during the active course of brucellosis. Ajlouni and Shaker (1998) reported that the patients with brucellosis have anemia due to bone marrow suppression, hypersplenism, autoimmune hemolytic, or alteration of iron metabolism, also added reduction in leucocytes count with relative lymphocytosis is common in brucellosis.

Akzdeniz *et al* (1998) showed hematological changes in a study performed for (233) patients with brucellosis, where recorded leucopenia in (21%), anemia (44%), and thrombocytopenia (8%).

In a study performed by Abdi-Liae *et al* (2005) for (85) patients infected with brucellosis in Iran to determine hematological changes during the active course of infection, where found leucopenia in (13.6%), anemia (43.5%), thrombocytopenia (12.5%) and pancytopenia in (2.4%).

A study performed by Behaz *et al* (2011) for (208) patients diagnosed with brucellosis in Iran to evaluate hematological changes found anemia

(46%) in males and (39.3%) in females, leucopenia (8.5%) and thrombocytopenia (12%) and pancytopenia in (1.5%).

Shi *et al* (2021) showed common hematological changes in a study performed on 109 patients infected with brucellosis in Anhui province in China, which included anemia (65.1%), leucopenia (24.8%), and thrombocytopenia (86.2%) with elevated in erythrocytes sedimentation rate in (86.2%) of all cases.

This study concluded that the brucellosis prevalence as a zoonotic disease among humans and the infection higher in females than males might be due to the females in rural area's direct contact and handling of livestock, which make the females highly exposed to the risk of infection with the animals and the animal products that use in cooking like (milk, meat, contaminated food, dairy product). The high incidence of brucellosis in the ages between (41-50) years followed by the ages between (31 -40) years, therefore the disease considers an occupational disease. Fatigue most common symptom followed by fever, arthritis, sweats, and

headache, while the lowest clinical signs and symptoms were weight loss, back pain, and anemia. The hematological analysis for the blood picture observed significant changes in leukocytes, Hemolytic anemia (hemoglobin), and abnormal account in Platelets also reported abnormal values in thrombocytopenia, lymphocytosis, and Neutrophiles with neutropenia in some cases infected with brucellosis in this study. Brucellosis may be considered the most common disease in the Missan governorate because some of the population tend to rear animals inside the houses, which demanded specific authorities to take care for control the disease, especially in the animals that consider the main source of the disease.

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