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RESEARCH ARTICLE

Impact of Brucellosis on Interleukin -23 Level, Acid Phosphates and Some Other Trace Elements

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Abstract

The study was carried out during a period of February 2017-July 2018 for the detection of brucellosis in 128 suspected patients with age group range from 17 - 69 years, who attended to Baghdad teaching hospitals, that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation. The diagnosis done by Rose Bengal test (RBT) method, a blood sample was taken from each patients as well as other 30 healthy control matching in age and gender. The study included measurement of the concentration of Interleukin-23, activity of Acidphosphatase, Copper and Zinc in sera of patients and healthy control. The result indicated that Rose Bengal test positive in 18 cases, 12 with a titer of 1:160 and 6 cases with a titer of 1:320. The Level of IL-23, Acid phosphatase increased significantly, with no statistical difference in copper and zinc concentration in both interval ages of patients sera in comparison with healthy control.

Keywords: Brucellosis , Interleukin-23, Acidphosphatase, Copper , Zinc.

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INTRODUCTION

Brucellosis is one of a bacterial Zoonotic illness that affecting animals and man worldwide[1,2,3]. Brucella species are cocco-bacilli gram-negative bacteria, they have many routes of transmission including: direct contact with animals or environment, consuming of uncooked animal product[4]. The transmission from person to person is rare[5,6], but they have been reported the disease transmission in association with Transfusion of blood and transplantation of bone marrow[5]. The Brucella infection in human either acute or chronic disease continue for many years[5,7]. There are four Brucella species that recognized as a human pathogens; *Brucella melitensis, Brucella abortus, Brucella canis and Brucella suis, with* their animal reservoirs of sheep and goats, cattle, dogs and swine respectively[6,7].

Brucellosis is a pyrogenic disease without specified symptoms, but it can cause localized effects in the organ systems[8]. Human Malta fever have an incubation period from five days to five months (2–3 weeks typically)[2,9]. Clinical symptoms vary widely from patient to patient and ranging from asymptomatic disease to severe illness but they may include recurrent fever, sweats mainly in the night, sleeplessness, arthralgia, headaches, fatigue, malaise, neuralgic symptoms and ache in joints, muscles , and-or back[9,10]. Some symptoms and signs can continue for a long time but others may not re-occur, including arthritis, recurrent fever, endocarditis, swelling of liver and-or spleen, swelling of testicles, neurologic symptoms (5% of all cases), and chronic fatigue (10) *Brucella* spp. are facultative intracellular pathogens which resist killing by neutrophils, replicate inside macrophages and in "non-professional" phagocytes and maintain a long lasting interaction with the host cells.

Therefore, host control of infection requires a set of cells and factors like CD^{4+} and CD^{8+} T lymphocytes, T-helper 1(Th1) type cytokines such as (IFNγ) and TNFα, and activated macrophages and dendritic cells (DC) which together promote a complex response against *Brucella*. It has been postulated that Th1 cytokines contribute to control Brucella infection[11]. Interleukin-12, chiefly a product of antigen-presenting cells (APC), is usually critical for the development of Th1 responses[12,13]. IL-10 is an anti-inflammatory cytokine secreted by T cells and macrophages. It interacts with the IL-10 receptor and like IFN-γ, signals through the Jack/Stat signaling pathway[14]. It is known to down regulate Th1 response during Brucellosis[15,16]. In this study the level of interleukin-23, Acidphosphatase and Copper with Zinc determined in patients with brucellosis in comparison with healthy control matched in age and gender especially Immunity and resistance to infection is affected by the nutritional status as Micronutrients such as Zn and Cu may affect and modulate immune responses.

MATERIALS AND METHODS

Studied groups

The study carried out during the period from (February2017- July2018), studied group were involved Suspected patients their age range between 17-69 years. Blood samples were obtained from a total of 128 patients clinically suspected with Brucellosis that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation.

Blood samples

Five mL of venus blood was obtained from each patients and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and then test for Brucellosis and stored in deep freeze at -20° C until the time for using. The current study included Immunological & Clinical biochemical aspects in comparison with healthy control.

Rose Bengal test (RBT)

RBT was carried out according to [17] with Brucella abortus S99 antigen (Spinreact SA, Gerona, Spain). Briefly, 30 of µl antigen was mixed on a white glossy ceramic tile, with an equal volume of sheep serum. The tile was then rocked at room temperature for 4 minutes and any visible agglutination and/or the appearance of a typical rim was taken as a positive result.

Immunological & Clinical biochemical tests.

The level of interleukin -23(IL-23) estimated by ELISA according to manual procedure of cusabio Biotech(Germany) was estimated by ELISA according to the manual procedure of

Creative – Diagnostic Company. Copper, Zinc and acidphosphatase Concentration determined according to manufactures instructions of Biosystem(Spain).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

RESULTS

Genders

According to their gender Patients with brucellosis, were studied, among them 10 were males and 8 were females. Males to females ratio was1.25 (Table 1).

Table 1. Distribution of Brucella patients according to gender and their percentage with RBT.

Patients	No.	Positive cases
Males	72(56.25%)	10 (55.55%)
Females	56(43.75%)	8 (44.45%)
Total	128(100%)	18 (100%)

Clinical study

Comparing the prevalence of different clinical sign and symptoms in brucellosis as clearly in (Table 2).

Table 2. Prevalence of different clinical sign and symptoms in Brucellosis cases.

Sign and Symptoms	No.	percentage
Fever	18	100%
Sweating	16	88.88%
Malaise	17	94.44%
Rigor	14	77.77%
Muscle pain	15	83.33%
Splenomegaly	9	50%
Hepatomegaly	10	55.55%
Lymph node enlargement	3	16.66%
Abdominal pain	2	11.11%
Loss of weight	10	55.55%
Headache	15	83.33%
Jaundice	1	5.55%
Vomiting	2	11.11%

Diagnosis of Brucella

The titer of 1:160 of antibodies to Brucella present in 12 cases with a percent of 66.66%, also, the titer of 1:320 of antibodies to Brucella present in 6 cases with a percent of 33.34% out of 128 cases in RBT method (Table 3).

Table 3. Distribution of Brucella patients according the antibodies titer with RBT.

Titer	No.	Percentage
1:160	12	66.66%
1:320	6	33.34%
Total	18	100%

Zinc and Copper

The concentration of zinc showed non significantly ($p \geq 0.05$) in both interval ages of patients with brucellosis in comparison with healthy control (Table 4). Also ,the result of copper statistically non significant in both interval ages of patients and healthy control.

Table 4. Zinc and copper concentration (mmol/l) in patients with Brucellosis and healthy control.

Parameter / Age categories	Zinc		Copper	
	Patients	Control	Patients	Control
17-36	9.4±0.3	10.2± 1.1	12.0±2.3	11.9±1.8
37-69	10.4±0.7	10.9±4.2	13.3±2.6	11.2±2.2

Acidphosphatase activity

The activity of acid phosphatase increased significantly ($pd \leq .05$) in both interval ages of brucellosis patients in comparison with healthy control (Table 5).

Table 5. Acid phosphatase activity in patients with Brucellosis and healthy control.

Patients Age Categories	Acid phosphatase(1U/ml)	
17-36	Patients	0.8 ± 0.2*
	Control	0.3 ± 0.2
37-69	Patients	0.7 ± 0.3*
	Control	0.5 ± 0.1

* $pd \leq 0.05$

Interleukin-23

The level of IL-23 Increased siginifigantly(pd≤0.05) in patients with Brucellosis in comparison with healthy control in both interval ages the value 327,388 pg ml for patients and 264, 268 pg ml for healthy control respectively (Table 6).

Table 6. Concentration of IL-23 pg/ml in patients with Brucellosis and healthy control.

Parameter Age Categories	IL-23(pg/ml)	
17-36	Patients	327+32*
	Control	264+18
37-69	Patients	388+16*
	Control	268+17

***** pd≤0.05

DISCUSSION

Brucellosis is a common Zoonotic disease of worldwide distribution. It continues to be a serious public health issue in Iraq, especially in epidemiological region in Iraq because population who consumes unpasteurized dairy products like milk, cheese and also population who contact with infected animals . diagnosis of brucellosis is only made after the causative organism is unexpectedly detected in cultures of blood or exudates specimens or serological test like: Rose Bengal test[18]. Although high percentage of false negative results is reported, the most common serologic test used in our country is Rose Bengal test (RBT). There is an association of direct and indirect laboratorial tests with clinical and epidemiological data is essential to perform a definitive diagnosis of brucellosis[19].

The incidences of brucellosis among males were more common than females (Table 1), although brucellosis affects both sexes (1, 8) and compatible with other reports[20,21]. high proportion of brucellosis cases with fever, brucellosis should be considered as a differential diagnosis for fevers of unknown origin. Arthralgia, myalgia, and back pain were common manifestations. The relative lower proportions of patients with vomiting and Jaundice compared to those reporting back pain might reflect limitations in diagnostic capacity (Table 2) the result in line with Dean *et al*(22) the result indicated no statistically difference in zinc and copper concentration in patients and healthy control serum (Table 4). The result compatible with(23)**.** Infection may also affect serum trace element concentration due to altered or impaired liver physiology or increased loss in the urine. In a general , Immunity and resistance to infection is affected by the nutritional status.

Micronutrients such as Zn and Cu may affect and modulate immune responses and thereby contribute to resistance to infectious diseases [24,25,26]. Acid phoshatase increased significantly in patients with Brucellosis (Table 5) in a general, ACP considered as a virulence factor in some pathogenic microorganism or may be important for management of disease severity[27] The increasing level of IL-23 may be produced mainly by activated antigen-presenting cells (APC) including dendritic cells (DC).

The activation of DC plays a pivotal role in shaping the immune responses. Following the detection of microbial products , for example via TLRs, activated DC can provide signals to prime naïve T cells to mount appropriate adaptive immune response .in a general, Lps of Gram-negative bacteria prime DC for enhanced IL-23 expression Via production of prostaglandin E2 to induced both the expression of IL-23p19 and IL-12p40 without affecting IL-12p35 expression in DC[28,29].

CONCLUSION

The result indicated that Rose Bengal test positive in 18 cases 12 with a titer of 1:160 and 6 cases with a titer of 1:320 . The Level of IL-23, Acid phosphatase increased significantly, with no statistical difference in copper and zinc concentration in both interval ages of patients sera in comparison with healthy control.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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