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RESEARCH ARTICLE

Estimated of auto antibodies levels as (Rheumatoid factor "RF" and antinuclear antibodies"ANA") in serum of suspected patients with Malta fever

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Abstract

Malta fever is disease mainly caused by *Brucella* sp. *as B. melitensis* and *B. abortus,* its prevalent on the Southern and Eastern of the Mediterranean basin, particularly in Tunisia, Libya, Egypt, Israel, Lebanon and Syria, and in the Arabian Peninsula and Iran.

Is a major zoonosis disease in the worldwide because its reservoir is a variety of domesticated animals, and the human infect results from direct or indirect contact with animal sources. The diagnosis of Malta fever depends on clinical features, and they must usually be supported by results of bacteriology and/or serology. Numerous serological procedures have been tried in the diagnosis of human brucellosis, the most widely used was the standard tube agglutination test, Rose -Bengal test and enzyme-linked immunosorbent assay. Recently, late stage of brucellosis may be inducing immune response to form of some auto-antibodies as rheumatoid factor (RF) and anti-nuclear antibodies (ANA).

This study was objective to evaluation the results of some immunological tests that used widely for diagnosis of human infection with Malta fever, Estimation of RF and ANA levels in serum of patient suspected, and determination the related them with Malta fever.

The results showed equal distribution to Malta fever occurs between male and female; 15 (62.5%) and, 16 (61.5%) respectively.

Also 15 (53.6%) are quality positive for RF compare to healthy control group with significantly differences ($P \leq 0.01$).

The results of this study showed 12 (66.6%) of infected patients were positively both of rose and RF tests, 12 (33.4%) were positively to rose test but negative to RF test, and 13 (34.6%) were positively to RF test but negatively to rose test compared to control healthy group with significantly differences ($P \leq 0.01$).

Additionally the estimation of ANA in patient serum specimens that were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) were showed highly levels of ANA in patients group compare to control group with highly significant value (P=0.01).

So when compare between immunological tests in this study, ELISA of ANA test was positive in 31 cases (62%), 12 cases (24%), and 25 cases (50%) were positive for RF and Rose tests respectively with significant and highly significant between them.

As a conclusion, the highly level of ANA with positively both of RF and rose tests may be indicate in diagnosis of brucellosis especially in late stage.

Introduction

Malta fever is a bacterial disease caused by bacteria infection known as brucella abortus [(1)]. Is also known as "undulant fever", "Mediterranean fever", is a type of zoonosis disease and the infection is almost transmitted either by direct or indirect contact with people in all age groups and both of gender, although there has been transmitted from infected animals or their products. It affects great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently transmission to the human population frequently occurs. It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, north and east of Africa, Middle East, south and central of Asia, and central and South America and yet it is often unrecognized and frequently goes unreported [(2)].

Patients with Malta fever has some group of symptoms such as irregular fever with variable duration, profuse sweating particularly at night, fatigue, anorexia, weight loss, headaches, arthralgia and generalized aching, epididymorchitis, osteoarticular disease (sacroilitis), endocarditic, local supportive infection of organs such as the spleen [(2)].

In the presence of symptoms, serology confirms the diagnosis. Rose Bengal test is usually used in serology is recommended for disease screening and in management of a patient with a finding of positive, in absence of symptoms with a positive test should be discussed with other inflammatory disease or other microbes infection, because Rose Bengal test is positive in 80% of patient and negative in 20% of patient [(3)].

Auto immunity and brucellosis is not clear and there are very limited publication about this interaction, Anti nuclear antibody could be found in the serum of a brucellosis patient during the active stage of disease because the Brucella can produce autoantibody agents intercellular nucleus, one of this antibody produced be Brucella patient is IgM that effected on joint and give false RF positive [(1)].

So this study is aimed to:

Determine of Rheumatic factor (RF) and Anti-nuclear antibodies (ANA) levels in serum of Malta fever patients group. Also determine the related between RF and ANA levels with Rose testes result in serum of suspected patients with Malta fever.

Materials and Methods

* **Selection the clinical cases:**

A total number of Malta fever patients were 50 include (24 male and 26 female), plus 20 healthy people as a control. Blood samples were taken in the period of time from October-2013/Febraray-2014.

* **Collection of blood specimens:**

A total volume of blood samples collected from each patient was (10ml) by vein puncture using disposable syringes. Blood was distributed into tubes and allowed to clot at room temperature for 30 min. and then centrifuged for 10 min. at 4000 rpm, then sera were separated and divided into several aliquots tubes, one of them used for serological tests (RF, and rose Bengal tests), but other tubes were frozen at -20o C, and thawed immediately prior to analysis of anti-nuclear antibody levels.

***Preparation of ANA test solutions:**

- **Prepare of working sample buffer**:

It was prepared by diluted 20ml of concentrated sample buffer with 80ml of sterile D.W., to obtained 1:5 of dilute working wash buffer.

- **Prepare of concentrated wash buffer (1X):**

It was prepared by diluted 20ml of concentrated wash buffer with 980ml of D.W., to obtain 1:50 of working wash buffer.

- **Prepare of working wash buffer:**

It was prepare by diluted 4ml of concentrated wash buffer (1X) with 196ml of sterile D.W., to obtained 1:200.

* **Diagnosis of suspected person infect with Malta fever by serological tests:**

- **Qualitative determine of Rose Bengal test (according to protocol of kit):**

Placed 50µl of serum, control positive, and negative onto the three selected ring of the slide, and added one drop of the Rose Bengal antigen onto serum sample, control positive and negative, by stirring stick mixed serum specimen, positive and negative control with Rose Bengal antigen.

Then rocked gently the slide for 2 min (automatic rotator can also be used), Observed agglutination may be appeared after min from beginning of shaking.

- **Qualitative determination of RF (slide latex agglutination) (according to protocol of kit):**

Placed 50µl serum specimen and control positive and negative onto the three selected ring of the slide, placed one drop of the RF antigen onto serum sample, positive, and negative control.

Then rocked gently the slide for 2 min (automatic rotator can also be used), Observed agglutination may be appeared after 1min from beginning of shaking.

* **Determination of Antinuclear Antibodies (ANA) levels in serum specimens suspected infect with Malta fever (according to Kit protocol):**

- Prepare of calibrators (standard proteins solutions):

Six of series vials contained 1.5ml of 0.3, 10, 30, 100, and 300IU/ml of standard protein solutions respectively were prepared and diluted according to manufacturing instruction and store at 4^{o}C until uses.

- Loading the samples into the micro-plate (according to Kit protocol):

One hundred µL of diluted serum from each patients was prepared by taking 10µl of serum and 1000µl of diluent sample buffer (1X) and mixed thoroughly to prepared 1:100 from diluted sample buffer, cut-off calibrator, calibrator (standard proteins), and negative/positive controls were added into the designed empty wells of the pre-coated well plate.

Incubated of micro-wells plate for 30min at 30^{o}C, washed the plate by added 300µl of diluted washing buffer to each well for 3time.

Added 100µl of conjugated enzymes solution into each well, Incubated plate for 30 min at 30^{o}C, and washed it by added 300µl of diluted washing buffer to each well for 3time.

Added 100µl of TMB substrate into each well and incubated plate for 30min at 30^{o}C, then added 100µl of stop solution into each well, and Incubated of plate for 5min. Read the absorbance of each well at 450nm within 30min.

The Results:

*** Distribution of studied groups according to gender:**

Table (1) was showed distribution of suspected patients with Malta fever group compared to healthy control group according to gender. Equal distribution occurs between male and female; 15 (62.5) and, 16 (61.5%) for male and female respectively.

*** Quality estimation of RF in studied groups:**

The result in the table (2) was showed out of Malta fever patients, 15 (53.6%) are quality positive for RF based onto positive agglutination; 8 (53.3%) and 7 (46.4%) for male and female respectively. Also the number and percentage of RF positive subjects out of healthy control are only 2 (10.0%), 1(50.0%) male and 1 (50.0%) females, respectively with significantly differences ($P \leq 0.01$).

*** Distribution of studied groups according to rose/RF agglutination result:**

Table (3) was showed the distribution of Malta fever patients group compare to control healthy group according to positively result both of rose test and RF. The results showed 12 (66.6%) of infected patients were positively both of rose and RF tests, 12 (33.4%) were positively to rose test but negative to RF test, and 13 (34.6%) were positively to RF test but negatively to rose test compared to control healthy group with significantly differences ($p \leq 0.01$).

*** Estimation of anti-nuclear antibodies (ANA) in studied groups:**

Some times Brucella infects can induce immune system to form ANA in serum specimens that were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) and the results were showed in the table (4). Highly levels of ANA in Brucella patients group (0.1994±0.00012, and 0.1381±0.0005 pg/ml) compare to control group (0.1087±0.0005, and 0.1083±0.0008 pg/ml), also increase in ANA level in female patients more than male patients compare to control group with highly significant value (p=0.01).

*** Determine of positively results for immunological tests to diagnosis of Brucella infect:**

When compare between immunological tests in this study, ELISA of ANA test was positive in 31 cases (62%) of acute infection with Brucella organism, Rose-Bengal test was positive in 12cases (24%), also RF was positive in 25 case (50%) with acute infection with significant and highly significant between them (table 5).

Table (1) Distribution of studied groups according to gender

Suspected patients group				Control group	
Gender	Infect	Un-infect	Total	Infect	Un-infect
Male	15 (62.5%)	9 (37.5%)	24	1 (11.1%)	8 (88.9%)
Female	16 (61.5%)	10 (38.5%)	26	1(9.1%)	10 (90.9%)
Total	31	19	50	2 (10%)	18(90%)

Table (2) Quality estimation of RF in Brucella patients group compare to healthy control group *								
	Patients group				Healthy control group			
Gender / RF	Male	Female	Total	Percent	Male	Female	Total	Percent
Positive	8	7	15	53.6%	1	1	2	10 %
Negative	4	9	13	46.4%	8	10	18	90 %
Total	12	16	28	100%	9	11	20	100%

Table (3) Distribution of studied groups according to Rose/RF agglutination result*							
RF agglutination result	Patients group			Healthy control group			
	Rose agglutination result			Rose agglutination result			
	positive	Negative	Total	positive	negative	Total	Percent
Positive	12 (66.6%)	13 (34.6%)	25	1	1	2	10 %
Negative	12 (33.4%)	13 (65.4)	25	8	10	18	90 %
Total	24 (100%)	26 (100%)	50	9	11	20	100 %

Table (4) Estimation of anti-nuclear antibodies levels in studied groups				
Gender	Mean ± SE of ANA level (pg/ml)		T value	P Value
	Patients group	Control group		
Male	0.1994±0.00012	0.1087±0.0005	3.999	0.001
Female	0.1381±0.0005	0.1083±0.0008	3.999	0.001

Immunological test	Positively case No. (%)	T value		P value
Rose test (1)	12 (24%)	(1) vs (2)	27.55	0.01*
RF test (2)	25 (50%)	(2) vs (3)	26.87	0.01*
ANA test (3)	31 (62%)	(3) vs (1)	27.54	0.01**

Discussion:

Diagnosis of brucellosis depends on clinical features with positive blood culture and raised Brucella agglutinins [4].

But, in the absence of culture facilities, the diagnosis of brucellosis traditionally relies on serological testing with a variety of agglutination tests such as the Rose Bengal test, the serum agglutination test (SAT), and the antiglobulin or Coombs' test [5,6]. This is mainly because the greatest incidence of brucellosis is found in underdeveloped countries with poor technical resources, as well as the fact

that it tends to occur in rural communities [7].

In this study, most cases of brucellosis should be diagnosed by serological tests, and most patients with acute brucellosis produce antibodies of immunoglobulin M (IgM) within few days of onset of the disease [8]. Also sometimes humeral immune response of the patient was produce auto-antibodies as RF and ANA to Brucella and self antigen, the agglutination test and ELISA test were used to diagnose this state [9].

In present study, Rose-Bengal test was positive in 12 (24%) by Spain kit significant differences. The most effective of this test is Rose-Bengal test which used as antigen suspension of smooth brucella cells stained with Rose Bengal and suspended in an acid buffer. The test discriminated against agglutinins of low avidity and is not subject to prozones [9]. Some investigators rely on the Rose-Bengal test, which has not been fully validated for human diagnostic use [9].

Results of this study showed that Anti-nuclear antibodies (ANA) were considered as a reason for false negative results of the serological tests. The reason for the adverse effects of ANA on the diagnostic yield of the agglutination tests might be related to structural similarity (molecular mimicry) between microbial and self-antigens [10]. Besides their structural similarity, auto- and blocking antibodies both appear to be consequence of working immune mechanisms that the test was more sensitive than agglutination test. Because the ELISA test was very sensitive and could as easily be made specific for antibodies [11]. Investigation from other studies of patients with acute brucellosis showed that the ELISA was the most sensitive diagnosis test [12].

The results of the present study was agreement with [13] who said in his reported; the patients with positive IgG ELISA results, signifying a late stage of the infection, showed positive ANA and Anti-ds DNA tests indicating presence of auto-antibodies while patient with IgM ELISA positive result, signifying a late stage of the infection, showed negative result for ANA test indicating absence of antinuclear antibodies.

Apparently from the results, autoimmune changes occur in the patients suffering from brucellosis, mostly in the late stage of infection.

Finally, the study shows that the ANA and Elisa tests were the best and accurate methods in diagnosis of the disease especially in late stage [13], followed by standard tube agglutination test for diagnosis of acute infection.

Conclusions:

This study was consulsed, the highly levels of ANA was found in serum patients suspect infected with Malta fever, also the RF and ANA are related with positively of rose test in Malta fever patients.

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