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ORIGINAL PAPER

Clinical Interpretation of Detection of IgM Anti-*Brucella* Antibody in the Absence of IgG and *Vice Versa*; a Diagnostic Challenge for Clinicians

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Abstract

Non-specific and often misleading clinical presentation of active brucellosis has made it a diagnostic puzzle for treating physicians. Clinicians rely greatly on the detection of IgG and IgM anti-*Brucella* antibodies by ELISA. Different patterns of positivity have been observed for IgG and IgM anti-*Brucella* antibodies in different cases, which further increases the risk of an erroneous diagnosis. Detailed herein is our two-years data with varied *Brucella* serology patterns and their clinical interpretation. Between January 2015 to December 2017, 1102 samples were processed in the Immunology Laboratory of KFHU for *Brucella* serology. 68 samples were positive for both IgG and IgM, 28 samples were positive for IgG and negative for IgM while 15 samples were positive for IgM and negative for IgG antibodies against *Brucella*. Electronic medical records, history of exposure, signs, symptoms, laboratory data, and the final diagnosis were recorded for all these patients. None of the patients with only positive IgM antibodies was finally diagnosed with brucellosis, while a diagnosis of brucellosis was established for only one patient with IgG antibodies positive in his serum. All the double-positive (IgG- and IgM-positive) serology patterns were diagnosed as having brucellosis. We concluded that determination of single IgM or IgG anti-*Brucella*-antibodies by ELISA could both be considered as definite and should ideally be interpreted in the context of appropriate clinical scenario and confirmation by other laboratory assays.

Key words: *Brucella*-specific IgG, *Brucella*-specific IgM, Brucellosis, ELISA for *Brucella*

Introduction

Human brucellosis is a common zoonotic infection and is still prevalent in many countries of Africa, Middle East, the Mediterranean area, Indian subcontinent, Central America and Central Asia (Papas et al. 2006). In the Middle East, the incidence of human Brucellosis was the highest during the 1990s, although a gradual decline in incidence has been witnessed afterward; still, Saudi Arabia is considered an endemic zone for Brucellosis. The clinical manifestation of the disease constitutes a broad range of signs and symptoms. Patients commonly present with fever, chills, fatigue, joint, muscle and back pain. The fact that symptoms are non-specific and can be shared by other infectious diseases makes it even more difficult for clinicians to diagnose it clinically.

Although the diagnosis is confirmed by isolation of the *Brucella* spp. from tissues or body fluids, the occupational risk of infection transmission to laboratory staff and the time-consuming and less sensitive culture examination has led to consider other diagnostic techniques more useful in the diagnostic workup of brucellosis.

Serologically, ELISA is the most popular and widely used diagnostic assay. *Brucella*-specific IgM antibodies are produced in the first week after the disease onset, reaching a maximum after two months. On the other hand, IgG antibodies are detected after the second week of infection, attaining a peak level of six to eight weeks later. While IgG response coincides more closely with the clinical course, the detection of specific IgM antibody in the absence of specific IgG antibody might be confusing for treating physician and therefore risks misdiagnosis of active brucellosis. Likewise, clinical interpretation of *Brucella*-specific IgG antibodies in the absence of IgM also creates confusion for clinicians.

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While some studies conducted on the diagnostic accuracy of ELISA mentioned a combined specificity of 100% for *Brucella*-specific IgM and IgG (Özdemir et al. 2011; Asaad et al. 2012), some other studies also highlighted the possible detection of *Brucella*-specific antibodies in cases without active brucellosis. Literature review reveals a great variation between studies performed on the sensitivity and specificity of *Brucella*-specific IgM and IgG antibodies detected by ELISA. Gomez et al. (2008) assigned a combined specificity of 100% for IgG and IgM and individual sensitivity of 60% and 84% for IgM and IgG, respectively. Mantur et al. (2010), on the other hand, reported a combined IgM and IgG ELISA specificity of 71.3% and a combined sensitivity of 100 %. Welch et al. (2010) found a combined specificity and a combined sensitivity of 55% and 92.3%, respectively. While anti-*Brucella* antibody detection does not always indicate brucellosis, a negative antibody profile does not exclude the infection, so results of *Brucella* serology should be interpreted with great caution (Welch et al. 2010).

Experimental

Materials and Methods

A retrospective search was performed on the serum samples that were analyzed by ELISA for the presence of IgG and IgM anti-*Brucella* antibodies at the Immunology Laboratory of King Fahad Hospital of the University (KFHU) from January 2015 to December 2017. Our hospital mainly serves eastern region population of Saudi Arabia and just like other provinces, overall incidence of brucellosis has decreased significantly in the eastern province. The ethical review board of KFHU approved the study protocol.

A total number of 1051 patients were evaluated for the presence of *Brucella*-specific antibodies. Out of these patients 512 (46%) were females and 590 (53%) were males. Mean age of the patient was 37 ± 12 years. Electronic files of these patients were reviewed for their detailed medical records including age, gender, previous history of exposure, signs, symptoms, the results of other laboratory tests to support the diagnosis of brucellosis (culture, rapid slide agglutination assay), and the number of times the serology was repeated for each patient. Final diagnosis, antibiotic treatment, and all other relevant clinical information were also recorded for the patients from progress and discharge notes of the treating physician.

IgG and IgM ELISA were performed using Abcam kits (Cambridge, UK; Cat #ab100547 and Cat #ab214568, respectively). All the steps were performed following instructions from the manufacturer. Sera and controls (50 µl) were dispensed to the antigen-coated wells of micro-test plates followed by first incubation for 60 min at 37°C. The wells were then washed followed by addition of anti-human IgG or IgM antibodies conjugated with an alkaline phosphatase enzyme. Later ELISA plates were incubated for another 30 min at 37°C and well were washed again. Enzyme substrate (50 µl) was then added to all wells followed by another incubation for 30 min and finally the stopping solution was added to all wells to inhibit the reaction. The color intensity was measured by ELISA reader.

IgG and IgM values above 11 standard units were considered positive. Values between 9 and 11 were considered uncertain while antibody levels below 9 standard units were considered negative for both IgM and IgG *Brucella* antibodies.

Blood culture was performed using a minimum of two culture set (one aerobic and one anaerobic) from two different venipuncture sites using a volume of 7–10 ml blood per vial for adult and 1–5 ml blood per vial for pediatric. All the vials were loaded to the Baltic system, which detected any CO_2 level change in blood culture vial as an indicator of the growth of the organisms. On identifying specific vial with CO_2 level change, subculture was performed for the sample using four plates (5% blood agar plate, Chocolate blood agar, MacConkey agar plate, and *Brucella* selective media).

RSAT (Rapid Slide Agglutination Assay Test) was performed using Atlas Medical Kits (Cambridge, UK; Cat #8.01.15.0.0010). All the steps were performed following instructions from the manufacturer. One drop (50 µl) each of serum and control was dispensed into separate circles on the slide test followed by one drop (50 µl) of the antigen into each well. Slides were placed on a mechanical rotator for one minute and agglutination was noted in bright indirect light.

Results

A total of 1102 samples were processed in the Immunology Laboratory of KFHU over a period of two years from January 2015 to December 2017 and ELISA serology was performed on them for IgM and IgG anti-*Brucella* antibodies.

Out of these samples, 991 were negative for both IgG and IgM antibodies, 68 samples were positive for both IgG and IgM, 28 samples were positive for IgG and negative for IgM while 15 samples were positive for IgM and negative for IgG antibodies against *Brucella*.

68 double-positive (IgG- and IgM-positive) samples belonged to 38 patients. The number of times serology was repeated for these patients and the number of times the result was the same (IgG and IgM positive) ranged between 2 and 5. The *Brucella* culture was

Table I
The serology patterns for *Brucella*-specific antibodies (ELISA).

Serology Pattern		Number of Samples	Number of Patients	Number of times serology was repeated with the same results
1	IgG^-IgM^-	991	976	1
2	IgG^+IgM^+	68	38	2–5
3	IgG^+IgM^-	28	22	1–5
4	IgG^-IgM^+	15	15	0

advised for all of these patients and turned out positive for 36 of them. RSAT was requested for only 13 patients and turned out positive for all of these double positive patients (Table I).

We have further divided single-positive patients into two groups. The first group included patients with serology positive for IgG and negative for IgM and the second group included patients who were positive for IgM and negative for IgG against *Brucella*.

Patients who had positive IgG and negative IgM anti-*Brucella* antibodies. The 28 samples positive for IgG and negative for IgM anti-*Brucella* antibodies belonged to 22 patients. The number of times per patient when the serology was IgG-*Brucella* positive and IgM-*Brucella* negative ranged between 1 and 5 (Table I). Of these patients, 81% (n = 18) were males while 19% (n = 4) were females. The age range of these patients varied from 7 to 64 years.

Of the 22 patients, two had additional risk factors of possible exposure to *Brucella* spp. other than residing in a *Brucella*-endemic country. One was a worker in a local slaughterhouse while other was a medical laboratory technologist. Both were residents of Dammam city, which lies in the Eastern province of Saudi Arabia.

Most common presenting complaint was fever followed by musculoskeletal pain. 12 patients presented with fever while five patients had joint-related symptoms that led the physician to request serology. Other symptoms leading to a serological assessment of *Brucella* antibodies included abdominal pain, pancytopenia, and dizziness.

RSAT test was requested for only two patients and the serology was negative for both. Blood culture for *Brucella* was performed for 21 out of 22 patients and turned out negative for all of them but one patient for whom the diagnosis of brucellosis was confirmed. Serology was requested for antinuclear antibodies (ANA) for all five patients with musculoskeletal symptoms. Four out of five patients demonstrated the presence of ANA in their sera. Anti-VCA-IgM and Monospot tests were positive in one patient and therefore a diagnosis of EBV-mononucleosis was established. Another patient was diagnosed as having HIV based on positive HIV serology on one occasion followed by a positive HIV-PCR. Hepatitis B surface antigen was detected in the serum of one patient that led to the final diagnosis of hepatitis B. In another patient, the diagnosis of syphilis was established after the demonstration of antibodies against *Treponema pallidum* in his serum.

The definite clinical diagnosis of these patients is presented in Table II. Out of 12 patients that presented with fever, the infectious cause was identified in seven with only one patient diagnosed with active brucellosis. Out of five patients with musculoskeletal pains, three were diagnosed as having connective tissue disease, while polyarthralgia and mechanical neck pain was a diagnosis in remaining two. Out of three patients with the symptoms of abdominal pain, one patient with accompanying fever was diagnosed as having syphilis, while the other two were diagnosed with self-limited abdominal pain and prostate malignancy. The diagnosis of lymphoproliferative disorder was established in one patient with pancytopenia. Stroke was the diagnosis in one patient with the weakness of limbs. No final diagnosis could be established in the remaining two patients who presented with dizziness and were treated symptomatically by the local physician.

Patients who had positive IgM and negative IgG anti-*Brucella* antibodies. All the 15 samples positive for IgM and negative for IgG belonged to 15 patients and serology was not repeated for these patients. The age of these patients at which serology was requested ranged from 14 to 40 years. 53% (n = 8) of these IgM-positive and IgG-negative patients were male, while 47% (n = 7) were females.

Other than the risk of living in a high prevalence country, one patient had an additional risk of working as a nurse in the infectious diseases clinic for two years.

Fever was the most common presenting symptom and the reason behind requesting Brucellosis workup in eight out of 15 patients. Joint pains became the second most common cause leading to request ELISA for anti-*Brucella* antibodies. Other causes leading to request *Brucella* serology included a cough associated with chest pain in two patients and hematuria in one patient. Only one patient presented with splenomegaly and abdominal pain.

RSAT was performed for 13 patients and turned out positive for four patients and culture was performed for 13 patients and turned out negative for all of them. The final diagnosis for each patient with IgM positive and IgG negative anti-*Brucella* antibodies is presented in Table III.

Not a single patient was diagnosed as having brucellosis. Of all the patients that presented with fever, one had influenza while another had syphilis as definite diagnosis after demonstration of positive the Influenza PCR and the Syphilis-Ig, respectively. Pyrexia of unknown origin (PUO) was diagnosed in two patients

Table II
Patients with positive IgG and negative IgM anti-*Brucella* antibodies.

	Age	Sex	Risk factors	Symptoms	Other diagnostic tests	Final diagnosis
1	48 yr	M	No	Arthralgia	Culture negative, ANA positive	Connective tissue disease
2	45 yr	F	No	Fever, knee joint pain	Culture negative, Rubella IgG positive, ANA positive	Connective tissue disease
3	59 yr	M	No	Paralysis (TIA)	Culture negative	Stroke
4	36 yr	M	No	Fever, cough,	Culture negative	Tuberculosis
5	31 yr	M	No	Fever and fatigue	Culture negative, Monospot positive, VCA-IgM positive	EBV – infectious mononucleosis
6	35 yr	F	No	Joint pain	Culture negative, ANA positive	Polyarthralgia
7	7 yr	M	No	Fever	Culture negative, RSAT negative	Meningitis
8	34 yr	M	Worker at slaughter--house	Fever	Culture positive, VCA-IgM negative	Brucellosis
9	35 yr	M	No	Fever, fatigue, malaise	Culture negative	PUO
10	45 yr	M	No	High-grade fever	Culture negative	Self-limited febrile syndrome
11	64 yr	M	No	Fever and weight loss	Culture negative, HIV-Ab positive	HIV
12	27 yr	M	No	Fever and low BP	Culture and RSAT negative	Septic shock of unknown origin
13	17 yr	M	No	Fatigue and dizziness	Culture negative	–
14	26 yr	M	No	dizziness	–	–
15	46 yr	M	No	Pancytopenia	Culture negative	Lymphoproliferative disease
16	17 yr	F	No	Joint pain	Culture negative, ANA positive	Connective Tissue Disease
17	31 yr	F	No	Fever	Culture negative	PUO
18	17 yr	M	No	Fever and abdominal pain	Culture negative, Syphilis-Ig positive	Syphilis
19	28 yr	M	No	Abdominal pain	Culture negative	Self-limited unspecified abdominal pain
20	44 yr	M	Medical laboratory Technologist	Generalized abdominal pain	Culture negative	Prostate malignancy
21	22 yr	M	No	Neck and right shoulder pain	Culture negative, ANA negative	Mechanical neck pain
22	32 yr	M	No	Low-grade fever	Culture negative, HBsAg positive	Hepatitis-B

with fever. In another two patients, the diagnosis of acute cystitis and spondylarthrosis was established. Tuberculosis and community-acquired pneumonia remained the final diagnosis in two patients that presented with a cough and chest pain. In four patients, the demonstration of antinuclear antibodies (ANA) led to the diagnosis of systemic lupus erythematosus (SLE). Cervicalgia was diagnosed in one patient who presented with shoulder pain while another patient with arthralgia was diagnosed with multiple sclerosis. One patient with hematuria as the chief presenting complaint was diagnosed as having renal stones.

Discussion

Saudi Arabia is considered a high prevalence zone for brucellosis and the prevalence is higher in a rural community as compared to urban areas. Non-specific presentation and a high index of suspicion on part of local physicians enabled us to describe a large series of patients who presented to the KFUH ID clinic with the clinical picture suggestive of brucellosis and variable patterns of serology results. Most common symptoms were fever and musculoskeletal pains.

Serology was performed by ELISA for all patients. Different patterns of positivity were observed for IgG and IgM anti-*Brucella* antibodies in these patients. Other laboratory assays that were performed to confirm the diagnosis included blood culture for *Brucella* and RSAT.

ELISA has a diagnostic advantage over other serological assays in an endemic setting where is a need to process a huge number of samples. However, sensitivity and specificity of IgG and IgM anti-*Brucella* antibodies have been a topic of debate in many studies (Gomez et al. 2008). Presence of IgM antibodies is indicative of acute infection but at the same time, IgM antibodies are well known for their cross-reactions with other bacterial species, like *Yersinia, Escherichia coli* O157*, Salmo-*

Table III
Patients with positive IgM and negative IgG anti-*Brucella* antibodies.

	Age	Sex	Risk factors	Symptoms	Other diagnostic tests	Final diagnosis
1	14 yr	F	No	Chest pain, cough and fever	Negative blood culture and RSAT	URTI
2	30 yr	M	No	Shoulder pain	Negative blood culture and RSAT	Cervicalgia
3	40 yr	F	No	Fever and arthralgia	Negative blood culture and positive RSAT	Acute cystitis
4	32 yr	F	No	Abdominal pain and splenomegaly	Negative blood culture and RSAT, ANA positive	SLE
5	22 yr	F	No	Arthralgia	–	Multiple sclerosis
6	37 yr	F	No	Fever and back ache	–	Spondylarthrosis
7	25 yr	M	No	Fever and body aches	Positive Syphilis-Ig, Negative blood culture and RSAT	Syphilis, HTN
8	17 yr	M	No	Myalgia and arthralgia	Positive ANA, Negative blood culture and RSAT	Connective tissue disease/SLE
9	31 yr	F	No	Arthralgia	Positive ANA and dsDNA, Negative blood culture and positive RSAT	SLE
10	24 yr	M	Nurse in a Medical Unit	Fever and fatigue	Negative blood culture and positive RSAT	PUO
11	30 yr	M	No	Fever and Myalgia	Influenza PCR positive, Negative blood culture and positive RSAT	Influenza
12	30 yr	F	No	Backache	Negative blood culture and negative RSAT, ANA positive	CTD/SLE
13	39 yr	M	No	Fever cough chest pain hemoptysis	Negative RSAT	Community acquired pneumonia
14	22 yr	M	No	Hematuria	Negative blood culture and negative RSAT	Renal stones
15	24 yr	M	No	Fever	Negative blood culture and negative RSAT	PUO

nella spp. and *Francisella tularenis (*Aranis et al. 2008). Since infections have been identified as the cause of fever in most of our patients, cross-reactions are probably responsible for the detection of IgM anti-*Brucella* antibodies in these patients.

Out of 53 patients who had IgM-antibodies in their sera, 38 were also positive for IgG and all of these double-positive patients were diagnosed for brucellosis based on suggestive clinical picture and isolation of the organism from the blood culture. None of the 15 patients who had only IgM antibodies against *Brucella* in their sera were actually diagnosed as having active brucellosis.

Furthermore, the other possible reason for false positive IgM antibodies could be the presence of rheumatoid factor (ISCIII 2018). Therefore, it is recommended to remove rheumatoid factor by pre-absorption before the determination of IgM anti-*Brucella* antibodies in sera to avoid possible interference with the result. One study has described a positivity of 8.8% for rheumatoid factor in patients with osteoarticular brucellosis (Corbel et al. 1985). Since most of the patients with IgM only antibodies in their sera presented with fever and joint-related symptoms and none of the sample was pre-treated to absorb rheumatoid factor, the false positivity of IgM antibodies can be attributed to interference due to rheumatoid factor.

Mantecón et al. (2006) have described IgG anti-*Brucella* antibodies more sensitive as compared to IgM in the diagnosis of brucellosis. Just like IgM antibodies, cross-reactivity leading to a false positivity has been described for IgG anti-*Brucella* antibodies. Binnicker et al. (2012) reported cross-reactivity of IgG with syphilis, while Varshoch et al. (2011) documented that tuberculosis might result in false-positive IgG antibodies. Similarly, in our series of patients, we found out a number of different infections leading to false-positive determination of IgG antibodies, including tuberculosis, syphilis, infectious mononucleosis, HIV and hepatitis B infections.

Only one patient with IgG only antibodies detected in his serum was diagnosed with brucellosis on confirmation by isolation of *Brucella* spp. from his blood culture. A possible explanation for the absence of IgM antibodies in this patient could be the fact that excess of IgG can lead to false-negative IgM in some immunoassays (Sharma et al. 2008). Al Dahouk et al. (2011) reported 11% of the patients with acute brucellosis to be negative for IgM antibodies.

The RSAT is considered a suitable screening test for the diagnosis of brucellosis; however, considering a great proportion of false-positive and false-negative results reported by RSAT, it is recommended to use a supplementary laboratory technique like ELISA or

MAT to further confirm the results of RSAT (Geresu et al. 2016). Four out of 13 patients with IgM only antibodies against *Brucella* in their sera were reported a positive by RSAT. Since none of these patients were diagnosed with brucellosis, the cross-reactions responsible for false-positive IgM were possibly leading to false-positive RAST results in these patients.

Different studies carried out on the sensitivity and specificity of IgG and IgM anti-*Brucella* antibodies reveal a great degree of variation. Furthermore, variability between the ability of different commercial IgM and IgG ELISA kits to diagnose brucellosis should be taken into account. A study conducted by Fadeel et al. (2011) evaluated the performance of four commercial kits for diagnosing brucellosis. Most of the investigation concluded the sensitivity of more than 90% for all kits with variable specificity. None of the kits obtained 100% diagnostic accuracy for diagnosing brucellosis. Authors further concluded that sensitivity of ELISA is increased when the levels of IgG and IgM against *Brucella* are considered in combination and that serology results should be interpreted in tandem with clinical history, symptoms of patients and other diagnostic tests. We found a sensitivity and specificity of 99% and 36%, respectively for *Brucella*-specific IgG ELISA. The individual sensitivity and specificity of *Brucella*-specific IgM were calculated to be 97% and 58%, respectively, when compared to bacterial culture. We further reported combined IgM and IgG ELISA specificity of 94% and a combined sensitivity of 98%, which is in accordance with above-mentioned studies (Welch et al. 2010) and therefore can help improve clinicians confidence in cases with double-positive (IgG^+/IgM^+) serology.

Being retrospective research, our study is subjected to some limitations. It was not possible to compare ELISA results with the MAT, the gold standard for serological diagnosis, to rule out false-positive and false-negative results for the determination of IgG and IgM anti-*Brucella* antibodies. Nevertheless, in our study, the false-positive results of IgM and IgG anti-*Brucella* antibodies may be supported by other diagnostic assays like blood culture and by taking into account the history and clinical course. Therefore, we believe that our results are in the clinical interest of the physicians who find it challenging to interpret different patterns of serology results by ELISA.

To conclude, the combined sensitivity of IgG and IgM against *Brucella* is higher when compared to individual sensitivity of IgG or IgM antibodies in the diagnosis of brucellosis. In case of positive IgM-only antibodies, the test should be repeated after preabsorption of the sample to remove rheumatoid factor. Our study highlighted the significance of cross-reactions leading to false-positive level of antibodies and therefore overdiagnosis of brucellosis in a region where medical conditions like tuberculosis, syphilis and connective tissue disorder can possibly simulate brucellosis. We further concluded that determination of IgG only or IgM only anti-*Brucella*-antibodies by ELISA should not be regarded as definite and should be interpreted in the context of appropriate clinical scenario and confirmation by other laboratory assays like MAT (Poester et al. 2010).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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