Jpn. J. Infect. Dis., 61, 366-370, 2008

Original Article

Assessment of Diagnostic Enzyme-Linked Immunosorbent Assay Kit and Serological Markers in Human Brucellosis

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(Received October 12, 2007. Accepted July 10, 2008)

SUMMARY: This study was performed to evaluate commercial brucella immunoglobulin G and M enzyme-linked immunosorbent assay (IgG and IgM ELISA) kits for the diagnosis of human brucellosis and to suggest a candidate prognostic marker for human brucellosis. We determined the serum levels of brucella IgG, IgM, C-reactive protein (CRP), soluble CD14 (sCD14), and neopterin in patients with brucellosis and compared them with those of normal healthy persons, patients with tuberculosis, and patients with other diseases. It was found that the sensitivity of ELISA to diagnose brucellosis was high when both IgG and IgM ELISA were used together. This study showed that serum CRP, sCD14, or neopterin levels were significantly high during the course of human brucellosis. The above markers, alone or in combination, might have the potential to evaluate treatment outcomes in human brucellosis. The markers that can predict the variability of agglutination titer was also determined. It was found that the titer value alone does not fully represent disease status.

INTRODUCTION

It is believed that the global incidence of brucellosis might be much higher than generally estimated. Brucellosis is endemic in Turkey, and the incidence of human brucellosis is increasing at alarming rates in this country. The problem is especially critical in the eastern part of Turkey, where the seroprevalence for brucellosis has reached 2.2% (1). In 2001, more than 15,000 individuals were infected with *Brucella* spp., with a population morbidity of 22.86/100,000. However it is believed that the actual prevalence is at least 50,000-100,000 per year, when unreported and subclinical cases are taken into account (2).

The key dilemmas confronted by physicians in brucellosis-endemic countries are the diagnosis and follow-up of patients with brucellosis. Molecular diagnostic analysis in a high-burden setting is not yet feasible due to high costs and the sophisticated infrastructure required. In most brucellosis-endemic countries, the standard agglutination tube (SAT) test is employed, together with consideration of the clinical signs and symptoms, to determine the diagnosis and prognosis of brucellosis. Agglutination tests are very sensitive and specific for the diagnosis of brucellosis (3). However SAT is cumbersome and time-consuming, and thus enzyme-linked immunosorbent assay (ELISA) may be an alternative which can be comparatively easily used in a large number of patients. Therefore, in the present study, we evaluated commercial ELISA kits which measure anti-brucella IgG and IgM for the diagnosis of brucellosis.

The criteria indicative of brucellosis cure have not yet been clearly defined, since antibody levels may remain elevated for an extended period of time after the end of treatment (4). Therefore, serum levels of innate immune markers may help to determine treatment outcomes. *Brucella* infection induces a Type 1 cellular immune response, as demonstrated by high levels of interferon (IFN)-γ in human serum (5,6). IFN-γ induces the production and release of neopterin from monocytes and macrophages (7). Neopterin levels have been determined in many infectious diseases (8,9) and are regarded as a biochemical marker of cell-mediated immunity (10). C-reactive protein (CRP) is an acute-phase reactant protein synthesized in hepatocytes. It is a sensitive marker of inflammation and tissue damage, and plays a role in eliminating bacteria (11). Soluble CD14 (sCD14) is a regulatory factor capable of modulating cellular and humoral immune responses by interacting directly with T and B cells, and it has been suggested to be an acute-phase protein (12). Several clinical studies have reported significant elevated levels of serum sCD14 under inflammatory conditions (12). Thus, serum levels of these might be a candidate prognostic indicator of human brucellosis. Therefore, in this study, we determined the serum levels of CRP, sCD14, and neopterin in patients with brucellosis and compared them with those of normal healthy subjects, and patients with tuberculosis and other diseases.

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PATIENTS AND METHODS

Diagnosis of brucellosis: To diagnose brucellosis, all serum samples were screened using the Rose Bengal Test (RBT), and when the titer was ≥1:40, the SAT test (Linear Chemi-

cals, S.L., Barcelona, Spain) was carried out to confirm the diagnosis. For SAT test serial twofold dilution of the serum was carried out to a dilution of 1:5,120 in order to avoid the prozone phenomenon. When the SAT titer was ≥1:160, diagnosis of brucellosis was made.

Serum samples: From May 2002 to April 2003, serum samples sent to the laboratory of the Department of Microbiology and Clinical Microbiology, Ankara Numune Education and Research Hospital, Ankara, Turkey, for the diagnosis of brucellosis were used in this study. These sera were collected from patients with suspected brucellosis who were seen at the same hospital. A total of 87 serum samples from patients with brucellosis and 13 samples from patients with diseases other than brucellosis were used in this study. The average age of the patients was 33.8 ± 17.1 (mean ± SD) years. Among the brucellosis patients, the number of females and males was 34 and 53, respectively. The group of patients with other diseases consisted of 8 females and 5 males. Since the present study was laboratory-based, the final diagnosis of illness in these patients remained unknown.

To compare the results of brucellosis-positive with brucellosis-free individuals, serum samples were collected from 20 normal healthy students and teachers at the Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey. There were 12 females and 8 males with an average age of 23.0 ± 5.6 years, and RBT and SAT were carried out to confirm that these individuals were free of brucellosis.

To compare the results of cases of disease in which the clinical presentation is difficult to differentiate from brucellosis, a total of 22 serum samples were collected from patients with tuberculosis treated at Ataturk Chest Diseases and Chest Surgery Central Education and Research Hospital, Ankara. Among these individuals, 20 were males and 2 were females, and their average age was 40.7 ± 12.6 years. RBT and SAT were carried out to rule out brucellosis in these patients. All patients were confirmed as having tuberculosis when at least 3 of the following 5 tests were affirmative: X-ray chest findings suggestive of tuberculosis, sputum microscopy showing acid-fast bacilli, sputum culture showing growth of *Mycobacterium tuberculosis*, positive tuberculin test with purified protein derivative, and a high erythrocyte sedimentation rate. All serum samples were kept at −80°C until use. Informed verbal consent was obtained from patients and healthy subjects.

Antibody assay by diagnostic ELISA: Serum levels of anti-brucella IgG and IgM were determined using ELISA kits according to the instructions of the manufacturer (Immuno-Biological Laboratories [IBL], Hamburg, Germany). The cut-off values recommended by the manufacturer were used to determine positive, negative, and borderline results representing cases of brucellosis, cases without brucellosis, and cases of unknown status, respectively.

Determination of serum levels of CRP, neopterin, and sCD14: Serum levels of CRP, neopterin, and sCD14 were determined by ELISA according to the kit manufactuer's instructions (IBL).

Statistical analysis: Statistical analysis was performed with Minitab 14 (Minitab Inc., State College, Pa., USA). The Anderson-Darling normality test was used to verify whether or not the data followed a Gaussian distribution. We found that the serum levels of markers in all groups (healthy subjects and patients with brucellosis, tuberculosis, or other diseases) had non-Gaussian distributions; therefore, the Mann-Whitney test was performed to determine whether or not differences observed among serum levels of the markers were statistically significant. To find out the correlations among different markers, the original serum levels of these markers were converted into Log_2 values and Pearson's correlation test was performed. A P value of less than 0.05 was considered as significant in all tests. Stepwise regression analysis was also performed to determine the factors (markers) predictive of SAT results variability.

RESULTS

Anti-brucella IgG and IgM ELISA for the diagnosis of brucellosis: Table 1 shows the results of anti-brucella IgG and IgM ELISA in serum samples from healthy individuals and patients with brucellosis, tuberculosis, or other diseases. The combined results of IgG and IgM ELISA (Table 1) were determined as follows: when one of the ELISA results was positive, the case was considered as positive, and when both of the ELISA results were negative, then the case was considered as negative. All borderline results were considered as negative; since such results did not give conclusive evidence regarding whether or not a patient was suffering from brucellosis, this confounded the outcome. Among the healthy subjects and patients with tuberculosis, all of the results were negative. One healthy sample and another sample from a patient with tuberculosis gave borderline results according to IgM and IgG ELISA, respectively; these cases were therefore considered as negative. The healthy subject

Table 1. Results of serum tested for brucellosis by commercial brucella immunoglobulin G and M enzyme-linked immunosorbent assay (IgG and IgM ELISA)

Subject category (no.)	IgG ELISA		IgM ELISA		Combined	
	Positive	Negative	Positive	Negative	Positive	Negative
Healthy humans (20)	–	20	–	20[1)]	–	20
Patients with brucellosis (87)	76	11[2)]	32	55[3)]	81	6
Patients with tuberculosis (22)	–	22[4)]	–	22	–	22
Patients with other diseases (13)	1	12	–	13	1	12

Borderline results were considered as negative.
[1)]: Number of bordeline results 1 sample. This individual did not have fever, malaise, headache or other sign-symptoms suggestive of brucellosis.
[2)]: Number of bordeline results 9 samples.
[3)]: Number of bordeline results 7 samples.
[4)]: Number of bordeline results 1 sample.

had no fever, malaise, headache, or any other signs or symptoms suggestive of brucellosis. As regards samples from patients with brucellosis, the IgG ELISA results were positive in 76 and negative in 11 cases; the IgM ELISA was positive in 32 and negative in 55 samples, and therefore the combined results were positive in 81 and negative in 6 samples. A total of 9 and 7 samples showed borderline results by IgG and IgM ELISA, respectively. These samples were considered as negative. In patients with other diseases, the IgG ELISA results were positive in 1 and negative in 12 cases; IgM ELISA was negative in all 13 samples, and therefore, the combined results were positive in 1 and negative in 12 samples. As shown in Table 2, the sensitivity, specificity, positive predictive value, and negative predictive value (13) of IgG ELISA were 87.4 (95% confidence interval 78.8 - 92.8), 98.2 (90.4 - 99.9), 98.7 (93.0 - 99.9), and 83.1 (72.2 - 90.3), respectively. For IgM ELISA, the sensitivity, specificity, positive predictive value, and negative predictive value were 36.9 (27.6 - 47.4), 99.1 (92.0 - 99.9), 98.5 (87.0 - 99.8), and 50.0 (40.9 - 59.1), respectively. The combined IgG and IgM ELISA results were determined according to the standard method (13), i.e., the sensitivity, specificity, positive predictive value, and negative predictive value were 93.1 (85.8 - 96.8), 98.2 (90.4 - 99.9), 98.8 (93.4 - 99.9), and 90.0 (79.9 - 95.3), respectively.

Serum levels of innate immune markers: A total of 80 serum samples from patients with brucellosis and all serum samples from the other groups were available for this investigation. Table 3 shows the serum levels of CRP, neopterin, and sCD14 for all four groups. The serum levels (mean ± SD) of CRP, neopterin, and sCD14 in the samples from patients with brucellosis (24.6 ± 27.7 μg/ml, 52.5 ± 47.1 nmol/ml and 8.6 ± 3.3 μg/ml) and tuberculosis (50.9 ± 44.9 μg/ml, 40.5 ± 45.5 nmol/ml and 10.2 ± 4.0 μg/ml) were significantly higher ($P < 0.0001$) than those of healthy individuals (0.8 ± 0.7 μg/ml, 3.8 ± 2.2 nmol/ml and 3.6 ± 0.8 μg/ml). The serum levels of CRP, neopterin, and sCD14 in the samples from patients with brucellosis and tuberculosis were significantly higher (P ranging from <0.05 to <0.0001) than those of patients with other diseases (10.8 ± 21.8 μg/ml, 14.5 ± 20.1 nmol/ml and 5.3 ± 2.0 μg/ml). The serum levels of neopterin and sCD14 in patients with other diseases were significantly higher ($P < 0.05$) than those of healthy subjects. The difference between serum CRP levels these latter two groups were not significant ($P = 0.496$). In patients with tuberculosis, significantly higher levels of CRP ($P < 0.005$) and sCD14 ($P < 0.01$) were observed, compared to those of patients with brucellosis. There were no significant ($P = 0.246$) differences between the serum neopterin levels of patients with tuberculosis and those with brucellosis.

Correlation between different markers: In patients with brucellosis, SAT test showed a significantly positive correlation with IgM ELISA (correlation coefficient = 0.355; $P = 0.001$), sCD14 (0.313; 0.005), CRP (0.271; 0.015), and neopterin (0.517; 0.000) levels. In these patients, serum neopterin levels also showed significant positive correla-

Table 2. The sensitivity, specificity, positive predictive value, and negative predictive value of IgG and IgM ELISA

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
IgG ELISA	87.4 (78.8 - 92.8)	98.2 (90.4 - 99.9)	98.7 (93.0 - 99.9)	83.1 (72.2 - 90.3)
IgM ELISA	36.9 (27.6 - 47.4)	99.1 (92.0 - 99.9)	98.5 (87.0 - 99.8)	50.0 (40.9 - 59.1)
IgG and IgM ELISA combined	93.1 (85.8 - 96.8)	98.2 (90.4 - 99.9)	98.8 (93.4 - 99.9)	90.0 (79.9 - 95.3)

Values in parentheses indicate 95% confidence interval.

Table 3. Serum concentration of CRP, neopterin, and sCD14 in healthy humans and patients with brucellosis, tuberculosis, and other diseases

	No. of subjects	CRP (μg/ml)[2),3),4)]	Neopterin (nmol/ml)[1),4),5)]	sCD14 (μg/ml)[1),5)]
Healthy humans	20	0.8 ± 0.7	3.8 ± 2.2	3.6 ± 0.8
Patients with brucellosis	80	24.6 ± 27.7	52.5 ± 47.1	8.6 ± 3.3
Patients with tuberculosis	22	50.9 ± 44.9	40.5 ± 45.5	10.2 ± 4.0
Patients with other diseases	13	10.8 ± 21.8	14.5 ± 20.1	5.3 ± 2.0

All values are expressed as mean ± SD.

1): $P < 0.05$. Serum levels of neopterin and sCD14 between healthy persons and patients with other diseases.

2): $P < 0.01$. Serum levels of sCD14 between patients with brucellosis and tuberculosis. Serum levels of CRP between patients with brucellosis and other diseases.

3): $P < 0.005$. Serum levels of CRP between patients with brucellosis and tuberculosis. Serum levels of CRP between patients with tuberculosis and other diseases.

4): $P < 0.001$. Serum levels of neopterin between patients with brucellosis and other diseases.

5): $P < 0.0001$. Serum levels of CRP, neopterin, and sCD14 between patients with brucellosis and healthy persons. Serum levels of CRP, neopterin, and sCD14 between patients with tuberculosis and healthy persons. Serum levels of sCD14 between patients with brucellosis and other diseases and patients with tuberculosis and other diseases.

Serum levels of CRP between healthy humans and patients with other diseases did not show significant difference ($P = 0.496$).

Serum levels of neopterin between patients with tuberculosis and brucellosis did not show significant difference ($P = 0.246$).

tions with IgM ELISA (0.323; 0.004), sCD14 (0.542; 0.000), and CRP (0.662; 0.000) levels. Furthermore, there was a significant positive correlation between CRP and sCD14 (0.446; 0.000) levels, whereas IgM and IgG ELISA results revealed a significant negative correlation (−0.225; 0.044).

Similar to patients with brucellosis, patients with tuberculosis had serum neopterin levels showing a significant positive correlation with serum levels of sCD14 (0.603; 0.003) and CRP (0.716; 0.000). A significant positive correlation (0.588; 0.004) was also seen between serum levels of CRP and sCD14.

Stepwise regression analysis was used to determine whether an observed titer could be predicted by any of the marker(s) of interest. When the analysis was carried out using data from the sera obtained from all subjects (healthy, brucellosis, tuberculosis, and non-brucellosis groups), it was found that serum levels of IgG, IgM, and neopterin (*P* ranging from 0.000 to 0.001; $r^2 = 61.5$) were predictive of variation in titer values in about 61% of the cases included in the analysis.

DISCUSSION

A number of researchers have advocated the use of ELISA for the diagnosis of brucellosis; however, this practive remains unpopular in endemic areas because, in many instances, the efficacy of ELISA over that of SAT test is not known in a particular setting. In this study, SAT test-positive sera were used to evaluate the results obtained with ELISA kits. A recent study revealed that the sensitivity of commercial IgG and IgM ELISA kits from different manufacturers were 91 and 100%, respectively (14). Similar to our results, low sensitivity using IgG and IgM ELISA tests was observed in another study that utilized kits from different manufacturers (15). In contrast to our study, the latter found that the sensitivity of IgM ELISA tests was higher than that of IgG ELISA tests (79.4 versus 45.6%); however, this discrepancy is not surprising, because in the latter study, serum from patients with *Brucella* bacteremia were used, and the IgM response can be higher than the IgG response (15). In line with findings reported by Gazapo et al. (16), we found that the combination of IgG and IgM ELISA results significantly improves sensitivity. Delays in diagnosis and insufficient treatment are responsible for a variety of different immunological patterns in brucellosis cases at the time of diagnosis (17). Therefore, the acute and chronic stages of brucellosis do not always appear as two distinct immunological entities; as a result, it is in many instances not possible to predict at which stage of disease a patient is presenting upon being seen by a physician. Therefore, for the diagnosis of brucellosis, the combined use of both IgG and IgM ELISA appears to be advantageous over application of either test alone.

CRP has been found to be a good prognostic indicator of acute brucellosis (18). However, in endemic situations, it is difficult to classify acute, chronic, and recurrent cases of brucellosis. In this study, we found that serum CRP, sCD14, and neopterin increased to high levels during brucellosis. A previous study also demonstrated that the sCD14 level is significantly high in human brucellosis (19). Our study revealed that in patients with brucellosis, serum levels of CRP, sCD14, neopterin, and also IgM significantly correlate with SAT results. Moreover, serum levels of all three innate immune markers (CRP, sCD14, and neopterin) were also positively correlated with each other in this study. Thus, one of these factors, or some combination thereof, may serve as a prognostic marker of human brucellosis.

Here, we also sought to determine whether serum levels of innate immune markers could account for the SAT titer variability associated with brucellosis. In this analysis, we found that IgG ELISA, IgM ELISA, and neopterin were significant predictors of titer. Moreover, the present findings showed that the IgG ELISA, IgM ELISA, and neopterin values paralleled the titer value. Approximately 39% of the variability in titer values remains unexplained, i.e., other factors might be involved, including the inherent error associated with titer measurement, severity of infection, and stage of disease (acute, chronic). Accordingly, it is possible that the titer value alone does not fully represent a patient's disease status. A wide range of titer values exists in patients with high IgM values. Indeed, high IgM values may be due to the high variability of titer values among brucellosis patients.

sCD14 is an immunoregulator which can inhibit in vitro cell proliferation and cytokine production (IL-2, IFN-γ, IL-4) by human T cells (20,21). In a previous study we observed a high level of IFN-γ in cases of human brucellosis; however, IL-2 and IL-4 were undetectable (6). One of the significant findings of the present study was higher serum levels of CRP and sCD14 in patients with tuberculosis than in those with brucellosis, which may indicate the presence of more severe tissue damage in patients with tuberculosis. In Turkey, brucellosis and tuberculosis are important causes of fever of unknown origin, and the unusual presentation of these diseases (22,23) renders clinical diagnosis difficult in highly endemic areas. Further study will be needed to determine whether these innate immune markers are suitable as prognostic indicators of human brucellosis, and whether or not high levels of CRP and sCD14 could be used to facilitate in the differential diagnosis of tuberculosis and brucellosis.

ACKNOWLEDGMENTS

We are grateful to the healthy human volunteers for providing sera. We thank Ozlen Konu for her cooperation and interest in this study. We also thank IBL (Immuno-Biological Laboratories, Ltd.), Turkey for providing us with the kits.

REFERENCES

1. Atmaca, S., Ozekinci, T., Akpolat, N., et al. (2004): Brucellosis seroprevalence in Southeast Turkey. Turk. J. Med. Sci., 34, 251-255.
2. Karabay, O., Sencan, I., Kayas, D., et al. (2004): Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: a randomized clinical trial [ISRCTNI 1871179]. BMC Infect. Dis., 4, 18.
3. Mert, A., Ozaras, R., Tabak, F., et al. (2003): The sensitivity and specificity of *Brucella* agglutination tests. Diag. Microbiol. Infect. Dis., 46, 241-243.
4. Ariza, J., Pellicer, T., Pallares, R., et al. (1992): Specific antibody profile in human brucellosis. Clin. Infect. Dis., 12, 131-140.
5. Diez-Ruiz, A., al-Amrani, M., Weiss, G., et al. (1993): Increased interferon-gamma and neopterin concentrations in patients with acute brucellosis. J. Infect. Dis., 167, 504-505.
6. Ahmed, K., Al-Matrouk, K.A., Martinez, G., et al. (1999): Increased serum levels of interferon-γ and interleukin-12 during human brucellosis. Am. J. Trop. Med. Hyg., 61, 425-427.
7. Huber, C., Batchelor, J.R., Fuchs, D., et al. (1984): Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J. Exp. Med., 160, 310-316.
8. Shi, M., Taylor, J.M., Fahey, J.L., et al. (1997): Early levels of CD4, neopterin, and beta 2-microglobulin indicate future disease progression. J. Clin. Immunol., 17, 43-52.
9. Millner, M.M., Franthal, W., Thalhammer, G.H., et al. (1998): Neopterin concentrations in cerebrospinal fluid and serum as an aid in differentiating central nervous system and peripheral infections in children. Clin. Chem., 44, 161-167.
10. Berdowska, A. and Zwirska-Korczala, K. (2001): Neopterin measurement in clinical dignosis. J. Clin. Pharm. Ther., 26, 319-329.

11. Mitaka, C. (2005): Clinical laboratory differentiation of infectious versus non-infectious systemic inflammatory response syndrome. Clin. Chem. Acta, 351, 17-29.
12. Bass, S., Gauthier, B.R., Spenato, U., et al. (2004): CD14 is an acute-phase protein. J. Immunol., 172, 4470-4479.
13. Guyatt, G., Sackett, D. and Haynes, B. (2006): Evaluating diagnostic tests. p. 273- 322. *In* R.B. Haynes, D.L. Sackett, G.H. Guyatt, et al. (ed.), Clinical Epidemiology: How To Do Clinical Practice Research. Lippincott Williams & Wilkins, Pa., USA.
14. Araj, G.F., Kattar, M.M., Fattouh, L.G., et al. (2005): Evaluation of the PANBIO brucella immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assay for diagnosis of human brucellosis. Clin. Diag. Lab. Immunol., 12, 1334-1335.
15. Memish, Z.A., Almuneef, M., Mah, M.W., et al. (2002): Comparison of the *Brucella* standard agglutination test with the ELISA IgG and IgM in patients with *Brucella* bacteremia. Diag. Microbiol. Infect. Dis., 44, 129-132.
16. Gazapo, E., Lahoz, J.G., Subiza, J.L., et al. (1989): Changes in IgM and IgG antibody concentrations in brucellosis over time: importance for diagnosis and follow-up. J. Infect. Dis., 159, 219-225.
17. Al Dahouk, S., Tomaso, H., Nockler, K., et al. (2003): Laboratory-based diagnosis of brucellosis–a review of the literature, part II: serological tests for brucellosis. Clin. Lab., 49, 577-589.
18. Navarro, J.M. (1990): C-reactive protein as a prognostic indicator in acute brucellosis. Diagn. Microbiol. Infect. Dis., 13, 269-270.
19. Ayaslioglu, E., Tekeli, E. and Birengel, S. (2005): Significant elevation of serum soluble CD14 levels in patients with brucellosis. Jpn. J. Infect. Dis., 58, 11-14.
20. Arias, M.A., Rey Nores, J.E., Vita, N., et al. (2000): Cutting edge: human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. J. Immunol., 164, 3480-3486.
21. Rey-Nores, J.E., Bensussan, A., Vita, N., et al. (1999): Soluble CD14 acts as a negative regulator of human T cell activation and function. Eur. J. Immunol., 29, 265-276.
22. Saltoglu, N., Tasova, Y., Midikli, D., et al. (2004): Fever of unknown origin in Turkey: evaluation of 87 cases during a nine-year-period of study. J. Infect., 48, 81-85.
23. Ergonul, O., Willke, A., Azap, A., et al. (2005): Revised definition of 'fever of unknown origin': limitations and opportunities. J. Infect., 50, 1-5.