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ORIGINAL ARTICLE

Relationship between Brucella immunocomplex and glomerulopathies

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ABSTRACT

Background: Brucellosis is a zoonotic disease with a very wide spectrum of clinical findings. Brucellosis is about 10 times more prevalent in patients with renal failure (dialysis patients) compared to population background. Precipitation of immunocomplexes produced by brucellosis is important in causing glomerulonephritis. Because the hallmark of glomerular diseases is abnormal protein loss in the urine we have decided to study proteinuria in brucellosis immunocomplex. The aim of this study was to evaluate probable relationship between brucellosis and glomerulonephritis.

Patients and methods: This cross sectional study, performed on 200 patients with a history of the disease for about 1 year, diagnosed as having chronic brucellosis. The diagnosis was confirmed in 150 patients and by applying Brucella Standard Agglutination Test (SAT) and 2-mercaptoethanol test (2-ME). Titers of IgG/IgM and IgG/IgA in two groups of "with proteinuria" and "without proteinuria" was measured.

Results: Both SAT and 2-ME tests test demonstrated that proteinuria increases with rising antibodies titers. In SAT, titer 1/160 was the most frequent, observed in 44% of the patients. In 2-ME test 1/40 titer was observed in 44% of the patients and was the most frequent.

Conclusion: Our results clearly demonstrated that in both chronic and acute patients, proteinuria increases with rising IgG/IgA and IgM/IgG titers. Therefore brucellosis can cause nephropathy but chronic or untreated brucellosis is more important because it can permanently damage kidney.

Keywords: *Brucellosis, Immune complex, Glomerulonephritis, Nephropathy.*
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INTRODUCTION

Brucellosis is an important zoonotic disease caused by a Gram-negative bacillus. It is a common health problem among people in close contact with livestock or living in rural areas with worldwide distribution. Four main Brucella species are capable of causing disease in human but most human infections are caused by Brucella melitensis (1), although it has a more limited geographical distribution than B. abortus (2,3). Brucellosis was first recognized in Iran in 1949. The disease has been endemic throughout the country (4). According to Ministry of Health in Iran, the annual reported incidence of human brucellosis in 2005 is 500,000 cases worldwide and more than 26000 cases in Iran.

Brucellosis cause reproductive failures in livestock and a severe multi-organ infectious

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disease in humans (5,6). One of the organs that can be affected by brucellosis is kidney and severe renal involvement in patients with systemic brucellosis has been reported (7, 8). It is shown that many infectious agents including Brucella can cause glomerulonephritis (8,9). In a retrospective study on the incidence of brucellosis in dialysis patients in the city of Isfahan we found that the incidence of Brucellosis is about 10 times higher in dialysis patients compared to incidence of Brucellosis in population background (10). A number of studies highlighted the role of immunocomplexes, such as antibodies produced by Brucellosis, in inducing kidney damage and glomerulonephritis (11). Glomerulopathies cause significant protein loss through the glomerules, resulting in nephrotic syndrome (12). In addition, glomerulopathies usually cause chronic renal failure due to progressive loss of functional nephrons (12,13). There is an overproduction of antibodies in recurrent as well as chronic forms of brucellosis. These antibodies could form immunocomplexes, which precipitate in renal interstitium, contribute to the chain of events resulting in renal insufficiency (12,13).

Specific IgM antibodies for brucella appear within the first week of the disease, make summit in 3 months, and then slowly decrease, and disappear (1). Even if IgM antibodies are progressively reduce and vanish from the blood, they sometimes may persist in low titers for a long time, being rarely detectable in high titers (2). The detection of Brucella-specific IgM antibody allows the diagnosis of patients with brucellosis at an early stage or acute disease and also may help to differentiate between patients in the early phase of brucellosis and those with chronic brucellosis (1,2,3). Seven to fourteen days after IgM antibody, IgG and IgA antibodies produce in human body against brucella (3). In some cases there is persistent elevation of IgG/IgA antibodies in association with chronic active infection. In other instances peak of IgG/IgA titers occurs after a phase of decline in concentration, suggesting a relapse of illness (1). IgA antibody is elaborated late and also may persist for very long intervals (12,13).

Interstitial nephritis and acute exudative glomerulonephritis are recognized renal complications of Brucellosis. Primary IgA nephropathy is an immunocomplex–mediated glomerulonephritis defined immunohistologically by the presence of glomerular IgA deposits accompanied by a variety of histopathologic lesions. Although primary IgA nephropathy receives the most attention, many other diseases are also associated with glomerular IgA deposits (12, 13). A causal relation between brucellosis and IgA nephropathy has been demonstrated (14,15). Although all IgG, IgM and IgA antibodies are produced in Brucellosis but most of the studies are focusing on effect of IgA and there is not conclusive evidence on importance of IgG and IgM in inducing nephropathy (15).

Patients suspected of having Brucellosis, are diagnosed by brucella serum agglutination test (SAT) and detection of IgM/IgG and 2-mercaptoethanol (2-ME) and detection of IgG/IgA antibodies, in the first 3 months or more. SAT has been extensively evaluated for reproducibility and accuracy and is still the most widely used test in serodiagnosis of human brucellosis (3, 10). It is demonstrated that although SAT is not as sensitive as PCR assay, but is as reliable as ELIZA and other commonly used methods (16). Treatment with 2-mercaptoethanol (2-ME) inactivates IgM antibody, and so the residual titer after 2-ME treatment indicates the anti-brucella IgG/IgA titers (17). In countries where the disease is highly endemic, a large proportion of the population may have persistent Brucella-specific IgG/IgA antibodies (18). Under such conditions, the detection of specific IgM antibodies is important to make the laboratory diagnosis of brucellosis in the early phase of the disease (18). The aim of this study was to evaluate probable relationship between chronic

brucellosis and glomerulonephritis. We also were interested in study of effects of different titers of IgM, IgG and IgA antibodies on proteinuria caused by this nephritis (18,19).

PATIENTS and METHODS

This cross sectional study was performed on 200 patients with a history of Brucellosis. They were referred to our Brucella laboratory in Faculty of Medicine, during years 2005 and 2006.

On all subjects proteinuria was measured. Because benign causes including fever, intense activity or exercise, dehydration, emotional stress and acute illness can cause proteinuria, all patients suspected with proteinuria caused by any of the above mentioned mechanisms were excluded from the study. Proteinuria usually is defined as a urinary protein excretion of more than 150mg per 24 hours urine specimen, and can be divided into trace, significant, heavy and nephrotic syndrome range proteinuria. In our study 24 hours protein of the urine was evaluated by calculating the ratio between creatinine to protein in random urine specimens (20). All of the patients had kidney ultrasound to determine the amount and degree of inflammation of renal tissues. Unfortunately, our patients refused to do renal biopsy, so determining exact kind of tissue injury was impossible.

The 2-ME test has proved useful in those patients with disease of more chronic onset, as well as those with more longstanding symptoms that may be due to brucellosis. A 2-ME agglutination of 1/20–1/40 would usually be indicative of active infection requiring treatment. However, there are no established criteria to indicate what constitutes a significant titer (18,19). In our study we also used SAT to diagnose brucellosis, similar to the majority of other studies (18).

SAT test does not discriminate between the IgG/IgA and IgM/IgG. In order to detect IgG/IgA alone, all sera were also tested by 2-ME SAT test as well. Treatment with 2-ME inactivates IgM antibody, and so the residual titer after 2-ME treatment indicates the anti-brucella IgG/IgA antibodies (18,19).

According to the test results, Brucellosis of the patients was confirmed and the patients without Brucellosis were excluded from the study. The samples were then divided into two groups of "with proteinuria" (test group) and "without proteinuria" (control group). The observed differences between two groups were statistically analyzed using t-student test.

RESULTS

The patients were between 15 to 74 years old and the highest prevalence of the disease was observed in age group 15 to 19 years old. Ninety of the patients were male and other 60 were female. The patients were referred to Ibne-Sina health center in Isfahan and were from Isfahan city or rural areas of Isfahan. Source of infection in urban patients were consumption of unpasteurized dairy products especially cheese and source of infection in rural patients were occupational as well as dairy products consumption. The average of proteinuria in our patients was between 500 to 600mg/24 hours, and the average ratio between creatinin to protein was 20.

The SAT and 2ME test results demonstrated that 150 of the patients were infected with Brucella, of which 50 had proteinuria (test group) but the rest of the patients (control group) had not.

SAT test was performed in titers between 1/80 and 1/640. An increase in proteinuria was observed with rising antibodies titers. The titer of 1/160 was the most frequent titer which was observed in 44% of the patients. Table 1 summarizes the results of SAT (IgG/IgM) test of the patients in both groups.

2-ME test was performed in titers from 1/10 to 1/160. In this test, similar to SAT test, an increase in proteinuria with rising of antibodies (IgG/IgA) titers was observed. The titer of 1/40, presented in

44% of the patients was the most frequent titer. The results of 2ME test are demonstrated in table 2.

Table 1. Seroprevalence of Brucellosis antibodies (IgM/IgG) in test group and control group (SAT test)

Titer	Test group	Control group
1/80 (n=60)	12 (24%)	48 (48%)
1/160 (n=51)	22 (44%)	29 (29%)
1/320 (n=33)	13 (26%)	20 (20%)
1/640 (n=6)	3 (6%)	3 (3%)
Total (n=150)	50 (100%)	100 (100%)

Table 2. Seroprevalence of Brucellosis antibody (IgG/IgA) in test group and control group (2-ME SAT)

Titer	Test group	Control group
1/10 (n=9)	1 (2%)	8 (8%)
1/20 (n=44)	7 (14%)	37 (37%)
1/40 (n=67)	22 (44%)	45 (45%)
1/80 (n=23)	15 (30%)	8 (8%)
1/160 (n=7)	5 (10%)	2 (2%)
Total (n=150)	50 (100%)	100 (100%)

In each titer of antibodies, the frequency rate of proteinuria was calculated in total number of patients having that titer. The results of this calculation demonstrated that parallel to rising titers we had more patients with proteinuria. These results are demonstrated in figure 1.

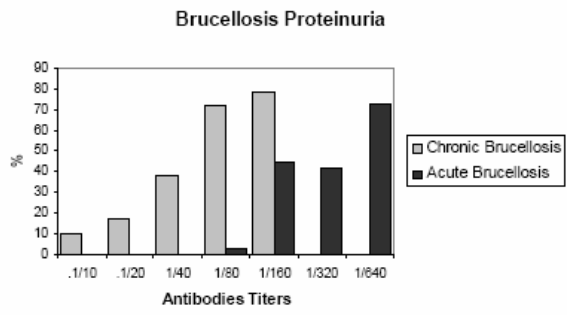

Figure 1. Frequency of proteinuria in patients affected with chronic and acute Brucellosis

DISCUSSION

The nephritis in Brucellosis is usually classified as three types: acute interstitial nephritis or pyelonephritis during the course of acute infection, chronic involvement with granulomas and caseification necrosis, and renal involvement in association with Brucella endocarditis. Secondary IgA nephropathy concomitant with Brucellosis has also been described. The underlying pathogenic mechanisms are either direct invasion of the bacteria (interstitial nephritis) or indirect glomerular involvement caused by the circulating immune complexes (glomerulonephritis). We believe that both mechanisms played a role in the pathogenesis in our patients (19).

Patients with Brucella glomerulonephritis almost always present with urinary sediment abnormalities, proteinuria, and/or azotemia. In our patients, gross hematuria, proteinuria, and increased BUN levels were detected during the initial laboratory analysis (19, 23).

Post-infectious glomerulonephritis (GN) is associated with bacterial agents and histologically appears most frequently as acute diffuse endocapillary or proliferative GN (21). Proteinuria is the primary clinico-pathologic sign of GN (22). Several infectious and inflammatory diseases including Brucellosis can cause GN (11,23). It has been shown that deposition or in situ formation of immune complexes may cause glomerulonephritis. The circulating immune complexes are also produced by Brucellosis (12,23).

Patients with Brucella glomerulonephritis almost always present with urinary sediment abnormalities, proteinuria, hypertension, macroscopic hematuria and azotemia (14). In addition to immune complex, Brucella bacteria also can induce cell mediated immunity producing granulomas in kidney cell (Brucelloma) (25).

Primary IgA nephropathy is an immune-complex–mediated glomerulonephritis defined immunohistologically by the presence of glomerular IgA deposits accompanied by a variety of histopathologic lesions (13,24), but as mentioned above unlike IgA, there is not conclusive evidence about the relationship between IgM and IgG, proteinuria and glomerulonephritis caused by Brucellosis.

Interestingly, it has been shown that brucellosis treatment can cure glomerulonephritis which is produced by Brucellosis, in early stages (when IgM has just appeared) and before irreversible renal failure (14,15).

In the present study we found that a great number of brucellosis patients with high titers of IgG/IgA and IgM/IgG antibodies also had proteinuria (table 1 and diagram 1). There was a close relationship between increase in these antibodies titer and increase in proteinuria ($P < 0.01$). So that the highest percentage of proteinuria (44%) was observed in 1/160 titer of these antibodies (IgG and IgM). It also should be noted that a decrease in proteinuria was observed above this titer (1/160) but this decrease was not significant.

Our results demonstrated that chronic brucellosis is more important in glomerulonephritis than acute brucellosis ($P<0.01$). However, as it can be observed on graph 1, it should be emphasized that in both chronic and acute forms proteinuria increases with rising brucellosis antibodies.

Post-infection glomerulonephritis is an immune complex mediated renal disease with significant participation of cellular immunity (21,23). Brucellosis may be an important cause of renal failure in endemic areas. This fact is often underestimated. Because the hallmark of glomerular diseases is abnormal protein loss in the urine we decided to evaluate proteinuria in brucellosis in relation with immunocomplexes. Our results clearly demonstrated that although both chronic and acute Brucellosis could cause glomerulonephritis but chronic or untreated brucellosis was more important because it could permanently damage kidney.

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