DETERMINATION OF PROCALCITONIN LEVELS IN BRUCELLA AND SALMONELLA BACTEREMIA

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ABSTRACT

Objective: *Elevated serum procalcitonin levels are of diagnostic value in most systemic bacterial infections, especially sepsis. However, there are insufficient data on the use of serum procalcitonin levels in the diagnosis of Brucella and Salmonella bacteremia. The aim of this study was to evaluate the diagnostic value of the serum procalcitonin test for Brucella and Salmonella bacteremia.*

Material and Method: *Thirty-one patients with Brucella bacteremia and 12 patients with Salmonella bacteremia were included. The control group consisted of 31 healthy blood donors. Procalcitonin levels were determined with the semi-quantitative BRAHMS PCT-Q test using sera from the control subjects and sera from patients obtained concurrently with blood or bone marrow sampling.*

Results: *No detectable procalcitonin (<0.05 ng/mL) was found in 15 (48.4%) of the 31 sera from patients with Brucella bacteremia. Another 15 (48.4%) had procalcitonin levels between ≥0.05 and <0.5 ng/mL. A serum procalcitonin level between ≥0.5 and <2 ng/mL was determined in one patient. Procalcitonin was at detectable levels in all the patients with Salmonella bacteremia. Levels were ≥0.05 and <0.5 in five patients, ≥0.5 and <2 ng/mL in five and ≥2.0 and <10 ng/mL in two. Five (16.1%) of the 31 control samples had procalcitonin levels between ≥0.05 and <0.5 ng/mL, while the remaining 26 (83.9%) had no detectable (<0.05 ng/mL) procalcitonin. Sensitivity and specificity of procalcitonin test for Brucella bacteremia were 3.23% [95% Confidence Interval (CI); 0.54-16.76], 100% (95% CI; 88.68-100), respectively; for Salmonella bacteremia were 58.33% (95% CI; 27.75-84.68), 100% (95% CI; 88.68-100), respectively, with a procalcitonin cut off level of ≥0.5 ng/mL.*

Conclusion: *Our results suggested that procalcitonin test is not sensitive enough in diagnosing Brucella bacteremia but it can be usefull in dismissing the disease. In Salmonella bacteremia the diagnostic value of the procalcitonin test is higher, and therefore the procalcitonin test can be used as a helpful method for diagnosing Salmonella bacteremia.*

Key Words: *Procalcitonin, Brucella, Salmonella, bacteremia* ***Nobel Med 2013; 9(3): 116-119***

BRUSELLA VE SALMONELLA BAKTEREMİLERİNDE PROKALSİTONİN DÜZEYLERİNİN BELİRLENMESİ

ÖZET

Amaç: Serumda prokalsitonin değerlerinin yükselmesi sepsis başta olmak üzere birçok sistemik bakteriyel enfeksiyonda tanısal değer taşımaktadır. Ancak Brusella ve Salmonella bakteremilerinde serum prokalsitonin düzeylerinin tanıya yardımcı bir belirteç olarak kullanılabilirliği ile ilgili yeterli veri bulunmamaktadır. Bu çalışmanın amacı Brusella ya da Salmonella bakteremisi bulunan hastalarda prokalsitonin testinin tanısal değerinin araştırılmasıdır.

Materyal ve Metod: Çalışmaya Brusella bakteremisi tespit edilen 31 hasta ve Salmonella bakteremisi olan 12 hasta dahil edildi. Kan dönörü olarak başvuran 31 sağlıklı birey kontrol grubu olarak alındı. Prokalsitonin düzeyleri kan kültürü veya kemik iliği kültürü ile eşzamanlı olarak alınan hasta serumları ve kontrol grubundan alınan serumlardan semikantitatif BRAHMS PCT-Q testi ile belirlendi.

Bulgular: Brusella bakteremisi olan 31 hasta serumunun 15'inde (%48,4) prokalsitonin değerleri tespit edilemeyecek düzeylerde (<0,05) idi. Bu örneklerin 15'inde (%48,4) prokalsitonin düzeyleri ≥0,05-<0,5 ng/mL değerleri arasında bulundu. Sadece bir hasta serumunda (%3,2) prokalsitonin düzeyi ≥0,5-<2 ng/mL değerleri arasında tespit edildi. Salmonella bakteremisi olan hastaların tümünde prokalsitonin düzeyleri tespit edilebilir düzeylerde idi. Prokalsitonin düzeyleri örneklerin 5'inde ≥0,05-<0,5 ng/mL, 5'inde ≥0,5-<2 ng/mL, 2'sinde ≥2,0-<10 ng/mL olarak bulundu. Kontrol grubundan elde edilen 31 serum örneğinin 5'inde (%16,1) serum prokalsitonin düzeyleri ≥0,05 - <0,5 ng/mL idi. Diğer 26 örnekte (%83,9) prokalsitonin varlığı gösterilemedi. Prokalsitonin sınır değeri 0,5 ng/mL olarak alındığında Brusella bakteremisi için prokalsitonin testinin duyarlılığı, özgüllüğü, sırasıyla %3 (%95 güven aralığı (GA); 0,01-0,19), %100 (%95 GA; 0,05-1); Salmonella bakteremisi için sırasıyla %58 (%95 GA; 0,29-0,84), %100 (%95 GA; 0,86-1) olarak bulundu.

Sonuç: Sonuçlarımız prokalsitonin testinin Brucella bakteremisi tanısında duyarlı bir test olmadığını, ancak bu testin hastalığın dışlanmasında yararlı olabileceğini düşündürmektedir. Salmonella bakteremisinde ise prokalsitonin testinin tanısal değerinin daha yüksek olduğu, bu nedenle Salmonella bakteremisi tanısında yardımcı bir yöntem olarak kullanılabileceği kanaatine ulaşılmıştır.

Anahtar Kelimeler: Prokalsitonin, Brusella, Salmonella, bakteremi **Nobel Med 2013; 9(3): 116-119**

INTRODUCTION

Brucella is a small, gram-negative, facultative intracellular bacterium that causes brucellosis in humans. Brucellosis is a multi-systemic infectious disease that can exhibit different clinical symptoms and signs. Since the disease generally proceeds with non-specific symptoms and findings, such as lethargy, lack of appetite, weight loss, muscle and joint pain, fever, hepatomegaly and splenomegaly, various laboratory tests are needed for diagnosis.[1] Growth of Brucella spp. bacteria in blood, bone marrow, biopsy specimens and/or cerebrospinal fluid is regarded as the golden standard for diagnosis of the infection.[2] However, culture sensitivity can vary depending on a number of factors, and particularly the stage of the disease and previous antibiotic use. In addition, since the bacteria grow slowly, the incubation of cultures may extend as long as 4-6 weeks.[3] Various serological tests, such as the Rose-Bengal plate agglutination test, standard tube agglutination test, Coombs' test or ELISA, can also be used for the diagnosis of brucellosis. However, problems such as antibody titer remaining high for a long time after healing, cross reactions arising with various gram-negative bacteria or false negative results stemming from an excess of antibodies, can also be encountered with these tests. The polymerase chain reaction (PCR) is another alternative technique that can be used in diagnosis, although this is not routinely employed for diagnostic purposes because of high costs and risk of contamination. It is more commonly used in brucellosis or local Brucella infections in which serological tests have proved inadequate.[2] Due to these difficulties in diagnosis, the search for different laboratory tests to support diagnosis in acute brucellosis continues.

Salmonella is another gram-negative, and facultative intracellular bacillus, one that causes gastroenteritis, septicemia and enteric fever. Diagnostic laboratory tests for Salmonella infections are also problematic. Laboratory diagnosis relies on the growth of the organism from blood or bone marrow followed by serological and biochemical identification of the bacteria. These procedures are time consuming (requiring a minimum of three days), expensive and require special equipment. Serological techniques such as the Widal test and enzyme immunoassays cannot establish a definitive diagnosis in Salmonella infections. Sensitivity is also low in PCR-based tests, due to low venous blood bacterial concentrations.[4]

Under physiological conditions, procalcitonin, a precursor of the hormone calcitonin, is synthesized by thyroid C cells and is generally at a level below 0.1 ng/mL in healthy individuals. Procalcitonin levels ≥0.5 ng/mL are of diagnostic value for most bacterial infections.[5] Procalcitonin production in various tissues of the body, in monocytes and granulocytes, has been shown in inflammatory processes such as sepsis, severe systemic bacterial infections, malaria and some fungal infections or autoimmune diseases.[6-12] Bacterial lipopolysaccharides and proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1), IL-2 and IL-6, have been shown to induce procalcitonin production in various cells.[5] Serum procalcitonin levels are frequently →

Table 1: Procalcitonin levels in the patient group with Brucella and Salmonella bacteremia and in the healthy controls

Procalcitonin value (ng/mL)	Brucella spp. (+) patient sera	Salmonella spp. (+) sera	Control group sera
<0.05 ng/mL	15 (48.4%)	0 (0%)	26 (83.9%)
≥0.05 - <0.5 ng/mL	15 (48.4%)	5 (41.7%)	5 (16.1%)
≥0.5 - <2.0 ng/mL	1 (3.2%)	5 (41.7%)	0 (0.00%)
≥2.0 - <10.0 ng/mL	0 (0%)	2 (16.7%)	0 (0%)
TOTAL	31 (100%)	12 (100%)	31 (100%)

used today for the diagnosis of a number of systemic bacterial infections, particularly sepsis, for differentiating between bacterial and viral diseases and for monitoring the success of antibiotic treatment.[13-15] However, there is little information in the literature regarding its potential use in diagnosis of Brucella and Salmonella infections.[16-18]

The purpose of this study was to determine the diagnostic value of procalcitonin level testing in serum samples obtained from patients with Brucella or Salmonella bacteremia.

MATERIAL and METHOD

Thirty-one patients with Brucella bacteremia and 12 patients with Salmonella bacteremia referred to the Karadeniz Technical University, Farabi Hospital with a preliminary diagnosis of brucellosis or salmonellosis between January, 2006, and March, 2011, were included in the study. Inclusion criteria of the study were positive blood or bone morrow culture for Salmonella spp. or Brucella spp.; and exclusion criteria were presence of signs of local infections, positive blood or bone morrow culture for other than Brucella spp. or Salmonella spp., severe trauma or surgical operation in last one week, in addition to malignancy or pregnancy. Thirteen (30.2%) patients were female and 30 (69.8%) male, with an average age of 35.7±30.8. Thirty-one controls consisting of nine (29%) women and 22 (71%) men applying to the Blood Center of Farabi Hospital as blood donors, with negative Rose Bengal plate agglutination and Widal plate tests and with an average age of 38.6±9.8 were enrolled as the control group.

An automated BACTEC 9240 (Becton Dickinson, Sparks, MD, USA) blood culture system was used for blood or bone marrow specimens. Five percent sheep blood agar, chocolate agar and eosin methylene blue (EMB) agar were added to blood culture containers giving a positive signal. Strains were defined as Brucella spp. or Salmonella spp. using standard microbiological techniques.[1]

Serum samples were received simultaneously with the blood or bone marrow culture in febrile period of the disease were used for procalcitonin testing. Serum samples were stored at -20°C until assay. Semi-quantitative BRAHMS PCT-Q (B.R.A.H.M.S. GmBH, Berlin, Germany) kits were used for determining the serum procalcitonin levels. Test packets were opened immediately before use, and 200 μL of serum samples brought to room temperature was placed in the pipetting area. Following incubation at room temperature for 30 min (in a maximum 45 min), the results were analyzed. Tests in which no control band developed were regarded as invalid. Band color intensity forming in the test area was compared with the intensities on the reference card and the results were analyzed semi-quantitatively.

RESULTS

Procalcitonin values were at undetectable levels in 15 (48.4%) of the 31 patients with Brucella bacteremia. Procalcitonin levels of ≥0.05 - <0.5 ng/mL were determined in another 15 (48.4%) patients. A level of ≥0.5 - <2.0 ng/mL was determined in one (3.2%) patient with Brucella bacteremia. Procalcitonin was at detectable levels in all patients with Salmonella bacteremia. Procalcitonin levels were ≥0.05 - <0.5 ng/mL in five patients (41.7%), ≥0.5 - <2.0 ng/mL in five (41.7%) and ≥2.0 - <10.0 ng/mL in two (16.7%).

Serum procalcitonin levels of ≥0.05 - <0.5 ng/mL were determined in serum specimens of five (16.1%) of the 31 specimens from the control group. No procalcitonin (<0.05 ng/mL) was determined in the remaining 26 (83.9%) patients (Table 1). Sensitivity and specificity of procalcitonin test for Brucella bacteremia were 3.23% [95% Confidence Interval (CI); 0.54-16.76], 100% (95% CI; 88.68-100), respectively, and for Salmonella bacteremia they were 58.33% (95% CI; 27.75-84.68), 100% (95% CI; 88.68-100), respectively, with a procalcitonin cut off level of ≥0.5 ng/mL.

DISCUSSION

In this study we measured procalcitonin levels in serum samples obtained from patients with bacteremia caused by Brucella spp. and Salmonella spp. Our most significant finding was that with the exception of one patient in the study group with active Brucella infection, serum procalcitonin levels were within the normal levels in all patients. The serum procalcitonin values of the control group were ≤5 ng/mL. Our search of the literature revealed no case-control study investigating the use of procalcitonin levels in serum in the diagnosis of Brucella. With this respect, our study may be the first of its kind. Some previous case reports have evaluated other blood values as well as procalcitonin in patients with Brucella infection. In agreement with our study, these have reported normal procalcitonin levels in such individuals.[16,17]

Brucella spp. differ from other gram negative bacteria with several respects. Brucella endotoxin is less toxic than that of produced by others. The organism is phagocytized by macrophages and monocytes after initial exposure. It can survive and replicate in phagocytic cells by inhibiting phagolysosome fusion, inactivating hydrogen peroxide

and superoxide by production of catalase and superoxide dismutase, preventing the release of toxic enzymes from intracellular granules, and suppressing the production of TNF-α. Phagocytosed bacteria are carried to some organs like spleen, liver, lymph nodes, bone morrow, and kidneys. The bacteria secrete proteins that induce granuloma formation in these organs. Therefore, procalcitonin levels not being elevated in these patients may be ascribed to Brucella spp. exhibiting a intracellular location and causing a lower level of secretion of certain cytokines, such as TNF-α, that induce procalcitonin production, and leading to granuloma formation.[5,19]

A procalcitonin level of ≥0.5 - <2.0 ng/mL was determined in one patient in this study. Although the presence of active Brucella infection was shown with Brucella spp. growth in bone marrow culture in this subject, examination of the records showed that the patient had presented to the emergency clinical with abdominal pain three days before specimen collection and had been diagnosed with acalculous acute cholecystitis and treated accordingly. The elevated procalcitonin level concerned may therefore have been due to acalculous acute cholecystitis.

Procalcitonin test seems to be specific but not sensitive for Brucella bacteremia. Because of the disease prevalence is very low, positive predictive value and negative predictive value were not given considering the results may be misleading. Procalcitonin values being within normal limits in almost all the patients with Brucella bacteremia suggests that the determination of serum procalcitonin can be useful for excluding the Brucella bacteremia.

Procalcitonin levels were ≥0.5 ng/mL in more than half of the patients (58.3%) with Salmonella bacteremia. This is a significant value for diagnosis of bacterial infections. There is limited number of studies on the value of procalcitonin level determination in the diagnosis of Salmonella infections.[18-20] Procalcitonin levels in bacterial gastroenteritis were investigated in one such study.[18] Eleven out of 15 bacteria isolated from stool cultures of patients in this study were Salmonella. The researchers reported that procalcitonin represented a good diagnostic test for bacterial gastroenteritis, with sensitivity of 40% and specificity of 92%.[18] Another study provided evidence of the capability of procalcitonin to directly neutralize the lipopolysaccharides of Salmonella spp., thus leading to a reduction of its major inflammatory mediators.[20]

Our results suggest that the procalcitonin test for Salmonella bacteremia was moderately sensitive and highly specific. On the other hand, the procalcitonin test may not be sensitive enough to be used as a supplemetary test in diagnosing Brucella bacteremia but it can be usefull in excluding the disease. However, our findings suggest that the procalcitonin test may be used as a helpful method for the diagnosis of Salmonella bacteremia.

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