Serum Levels of Interferon-γ, Interleukin-12 and Interleukin-8 in Patients with Brucellosis

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الخلاصة

خلفية الدراسة: مرض حمى مالطا هو أحد أكثر الأمراض البكتيرية الشائعة المشتركة بين الأنسان والحيوان ، وتلعب السايتوكينات المختلفة دورا مهما في امراضية البكتريا المسببة لهذا المرض. ويعد الأنترفيرون گاما والأنترلوكين12 من أهم السايتوكينات الضرورية للتخلص من اللإصابة بالبروسيلا.

أهداف الدراسة: هدفت هذه الدراسة الى قياس المستويات المصلية لكل من الأنترفيون گاما والأنترلوكين12 والأنترلوكين8 والإرتباط بين هذه السايتوكينات في المرضى المصابون بحمى مالطا.

المواد وطرائق العمل: شملت الدراسة 60 مريضا بحمى مالطا بالإضافة الى 26 شخصا سليما ظاهريا مثلوا مجموعة السيطرة. جمعت عينات دم من جميع أفراد الدراسة إذ تم فصل مصل الدم من هذه العينات ، وأستخدمت طريقة الإدمصاص المناعي الإنزيمي لتقدير المستويات المصلية للأنترفيون گاما والأنترلوكين12 والأنترلوكين8 .

النتائج : أظهرت المستويات المصلية لكل من الأنترفيون گاما والأنترلوكين12 ولكن ليس للأنترلوكين8 أرتفاعا معنويا في المرضى مقارنة مع مجموعة السيطرة . فضلا عن ذلك فقد أظهرت النتائج أن مستويات الأنترفيون گاما قد ارتبطت ايجابيا مع مستويات الأنترلوكين12 وسلبيا مع مستويات الأنترلوكين8 .

الإستنتاجات : كان لكل من الأنترفيون گاما والأنترلوكين12 دورا حاسما في مناعة وإمراضية البروسيلا ، ويمكن إستخدام الستويات المصلية لهذين السايتوكينين كمؤشرين لوجود إصابة مزمنة بالبروسيلا.

الكلمات المفتاحية : حمى مالطا ، أنترفيون گاما ، أنترلوكين12 ، أنترلوكين8 ، سايتوكينات

Abstract

Background: Brucellosis is one of the most common bacterial zonosis. Different cytokines have an important role in the pathogenesis of the causative bacteria. Interferon-γ (IFN-γ) and interleukin-12 (IL-12) are among the most essential cytokines required for the elimination of infection with *Brucella.*

Aims: The study aimed to measure serum levels of IFN-γ, IL-12 and IL-8 and correlation among these cytokines in patients with brucellosis.

Subjects and Methods: A total of 60 patients with brucellosis and 26 apparently healthy controls were enrolled in this study. From each participant, blood samples were collected from which serum was obtained. Enzyme-linked immunosorbent assay was used to estimate serum levels of IFN-γ, IL-12 and IL-8 in each serum sample.

Results: Serum levels of IFN-γ and IL-12 but not IL-8 were significantly higher in patients with brucellosis compared to control. Furthermore, serum levels of IFN-γ correlated positively with IL-12 and negatively with IL-8.

Conclusion: Both IFN-γ and IL-12 have a crucial role in the immunity and pathogenesis of *Brucella* and can be used as indication for chronic infection.

Keywords: Brucellosis, IFN-γ, IL-12, IL-8, cytokines

Introduction

Brucellosis is a systematic disease in human that can involve almost any organ in the body (1). With over 500000 new cases annually, this disease is the world's most common bacterial zonosis. The Middle East, including Iraq, is an endemic area, and several countries in the region reported the world's highest incidence of the disease (2).

Three predominant *Brucella* species are seen frequently in human infection: *B. melitensis, B. abortus and B. suis.* Infection with *B. melitensis* was more frequently recorded and seems to be associated with more severe illness compared to the other two species (3).

Brucella possess many mechanisms that enable them to evade immune system. At early stage of infection, these bacteria can elude the recognition by toll-

like receptor 4 by virtue of production of Vi antigen which creates a capsule around the lipopolysaccharide (LPS)(4). Furthermore, the intracellular residence of *Brucella* inside the macrophages protects them from many effective components of the immune system.

Cytokines appear to have an important role in the pathogenesis of brucellosis, and the Th1/Th2 balance may involve in the susceptibility or resistance to the disease (5). As the pathogen is intracellular, Th1 and its related cytokines are responsible for control of the infection. Experimental studies showed that IFN-γ is essential for elimination of *Brucella* and for host survival in case of virulent *Brucella* challenge (6). Interleukin-12 has been shown to play significant role in controlling infection through the activation different immune cells such as natural killer (NK) cells (7). Ahmed *et al.* (1999) reported elevated serum levels of both IL-12 and IFN-γ in patients with brucellosis compared with control (8). Interleukin-8 is an important pro-inflammatory cytokine, and an *in vitro* studies showed that human osteoblasts and hepatocytes infected with *Brucella* respond with a limited secretion of this cytokine (9,10). Furthermore an increased in serum levels of IL-8 levels in patients with brucellosis were recorded by some workers (11,12). This study aimed to estimate serum levels and correlation among IFN-γ, IL-12 and IL-8 in patients with brucellosis.

Subjects and Methods

Patients

A total of 60 patients (47 Male and 13 female; age range 32-80 years) with brucellosis who attending Al-Yarmook Teaching Hospital/ Baghdad from September 2013 to January 2014 were enrolled in this study.The diagnosis criterion was serum agglutination titers ≥1/160 in the presence of compatible clinical picture including fever, night sweat, anorexia, weakness, malaise and arthralgia. Another 26 apparently healthy individuals (21 male and 5 female; age range 28-59 years) were recruited as control group.

From each participant, five milliliters of venous blood was obtained which was left to coagulate, and then serum was separated by centrifugation.

Immunological Assays

Enzyme-linked immunosorbent assay (Diagnostic Automation Inc, USA) was used to measure the concentration of IFN-γ, IL-12 and IL-8 in the serum samples using ready kits (Immunotech, France) according to manuals supplied with the kits. Briefly, exactly 25µl of standards, specimens and controls were added into 6, 60 and 26 allocated wells of ELISA microtiter plate respectively. For each well, 100µl of enzyme conjugate was added, mixed for 30sec and incubated at room temperature for 60min. The plate was rinsed 5 times with distilled water and then attached to a filter paper to remove all the residual water droplets. The TMB solution (100µl) was added into each well, mixed for 5sec and incubated at room temperature for 20min without shaking. The reaction was stopped by adding 100µl of stop solution to each well and mixed for 30sec. The optical density was read at 450nm within 15min. The apparatus is equipped with software that can read the concentrations directly without need for absorbance values and standard curve.

Statistical Analysis

Statistical package for social sciences (SPSS) software was used to analyze the data. T-test of independence was used to find out the significant differences between means of the two groups. Interleukins concentrations in the serum were expressed as means± standard deviation (SD). Statistical significance was set at a P value≤ 0.05.

Results

Interferon-γ

Patients with brucellosis had significantly higher serum levels of IFN-γ (183.294±80.837 pg/ml) compared to control group (44.937±30.971 pg/ml)(t=8.439, *P*<0.001)(Figure 1).

Interleukin-12

Mean serum concentration of IL-12 in patients and control were 280.47±144.239 pg/ml and 97.836±55.259 pg/ml respectively with highly significant difference (t=6.243, *P*<0.001)(Figure 2).

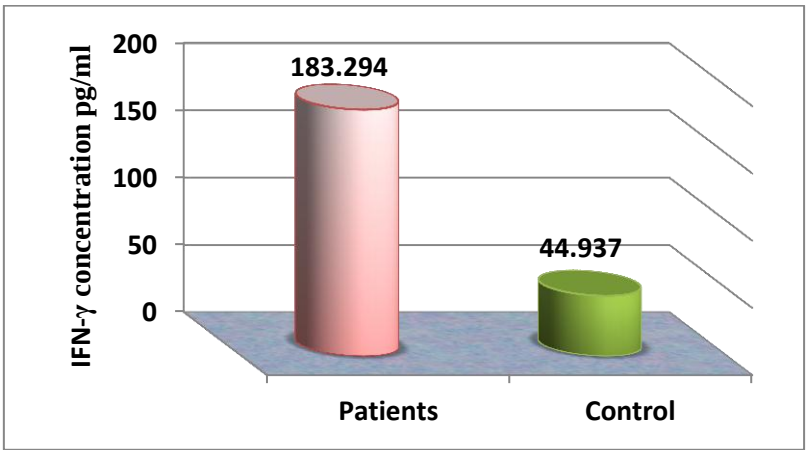

Figure 1: Serum concentration of IFN-γ in patients and control

Interleukin-8

Figure 3 shows mean serum concentration of IL-8 in brucellosis patients and control. Patients had relatively not higher serum concentration of this cytokine (51.519±35.681 pg/ml) than control group (42.448±36.185 pg/ml) but the difference was insignificant (t=1.018, *P*=0.368).

Correlation among the Cytokines

Pearson correlation test among the three cytokines in patients with brucellosis revealed positive highly significant correlation between IFN-γ and IL-12 (r=0.394, *P*=0.02), whereas, there was a negative significant correlation between IFN-γ and IL-8 (r=-0.257, *P*=0.047). Interleukin-12 also showed negative correlation with IL-8, however, this association was not significant (r=-0.154, *P*=0.241) (table 1).

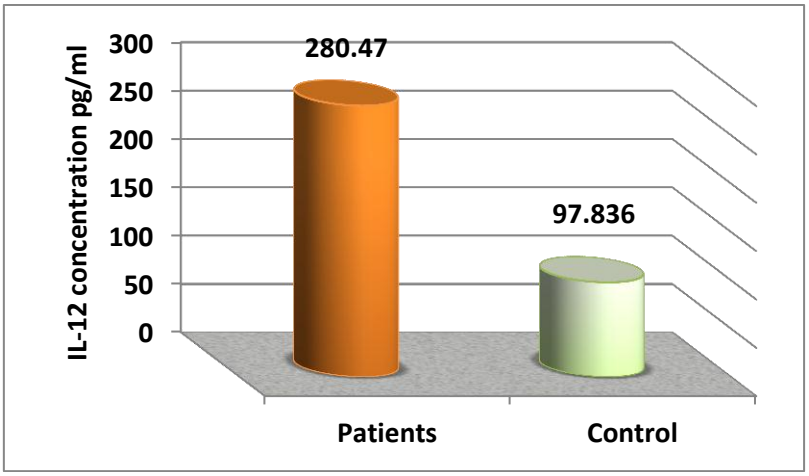

Figure 2: Serum concentration of IL-12 in patients and control

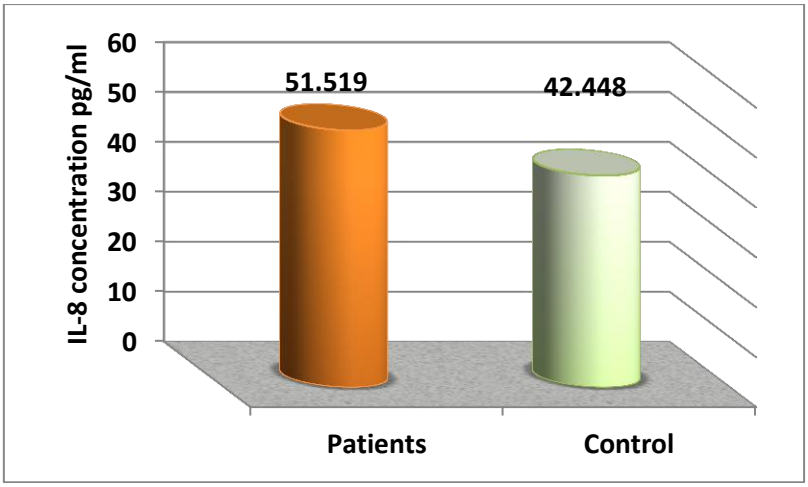

Figure 3: Serum concentration of IL-8 in patients and control

Table1: Correlation among IFN-γ, IL-12 and IL-8 in Patients with Brucellosis

		Interferone	Interleukin12	Interleukin8
Interferone	Pearson Correlation	1	.394**	-.257*
	Sig. (2-tailed)		.002	.047
	N	60	60	60
Interleukin12	Pearson Correlation	.394**	1	-.154-
	Sig. (2-tailed)	.002		.241
	N	60	60	60
Interleukin8	Pearson Correlation	-.257*	-.154-	1
	Sig. (2-tailed)	.047	.241	
	N	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Discussion

Host protection against *Brucella* depends on CMI involving mainly activated professional antigen-presenting cells (APCs), Th1 cells and CD8+ cytotoxic T lymphocyte (13). These cells exert their activities in different ways among which is the production of cytokines. The most common cytokines involved in the immunity against infection with *Brucella* are IFN-γ and IL-12. In this study, both of these cytokines were found to have high serum levels among patients with brucellosis compared with control. These results are in accordance with many previous studies (8; 11; 14;15) who reported elevation in one or both of these cytokines in brucellosis patients.

Most studies indicated that CD4+ T lymphocytes are the major producer of IFN-γ although other subsets such as CD8+ Tlymphocytes, γδ T lymphocytes and NK cells also participate in the production of this cytokine (16; 17). As there are relatively high serum levels of IFN-γ, it indicates an increased number of CD4+ which, in turn, indicates a chronic infection (13).

One of the most important innate immune response against intracellular

microbial infection is the secretion of IL-12. It is a regulatory cytokine that connects innate and adaptive immune response. This cytokine exerts its effect mainly through induction of INF-γ production (18) through activation of CD4+ and NK cells (7). Therefore it is reasonable to assume that high serum levels of INF-γ implies a parallel increased in IL-12 levels. In fact, this is evidenced not only by the significant high serum levels of the two cytokines, but also by the presence of significant correlation between them.

The elevated serum levels of IFN-γ and IL-12, although it involves active immune response, indicates that the causative pathogen still active and the immune response in its innate and adaptive arms did not eliminate the infection completely. Thus, besides the clinical symptoms, serum levels of these two cytokines can be used as an indication for the presence of active chronic brucellosis.

Interleukin-8 is produced by a variety of cells in response to LPS and proinflammatory cytokines. The main role of this cytokine referred to its activity as a potent chemoattractant and activator of monocytes, lymphocytes as well as neutrophils (19). The present study revealed no significant elevation in serum levels of this cytokine in brucellosis. In their study on acute brucellosis, Akulut *et al.* (2007) observed no change in serum level of this cytokine, while Refik *et al.* (2004) reported high IL-8 levels in brucellosis patients compared with control (14;11). Furthermore, Demir and Ural (2012) found significantly high IL-8 in relapsed brucellosis patients and suggested to monitor serum level of this cytokine to assess in predicting brucellosis relapse (20).

It seems that this cytokine is mostly associated with acute infection (21), and it is serum levels decline in chronic phases. This concept was supported by a study of Nejad *et al.* (2011) who found an increased and normal serum levels of IL-8 in acute and chronic toxoplasmosis respectively (22). Although the causative agent in the last study is a protozoan parasite, it shares many properties with *Brucella*, the most important of which are intracellular residence and infection of various types of host cells.

Body cytokines interact with each others, and many cytokines act as a feedback signals to reduce the production of other cytokines. There is no evidence for the negative effect of IL-8 on the production of IFN-γ, but the reverse has been proven. Cassatella *et al.* (1993) *in vitro* demonstrated that IFN-γ suppressed IL-8 production by polymorpho-nuclear leukocytes even when these cells were stimulated with different stimulants such as LPS and TNF(23). This explains the negative correlation between IFN-γ and IL-8.

In light of these results, it can be concluded that IFN-γ and IL-12 are the main cytokines in immunity against infection with *Brucella* and continuous elevation of these cytokine may indicate the chronic phase of the disease.

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