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Serum Levels of Interferon Gamma in Patients with Brucellosis in a Saudi Hospital

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Authors' contributions

All the authors have made substantial contributions to the intellectual content of the paper. The author MAAS planned and designed the study, wrote the protocol, participated in the interpretation of the results and analysis, drafted and critically revised the manuscript for important intellectual content and approval of the version to be published. Author AIHAH collected the data, performed the practical laboratory activities, and participated in the interpretation of the results and drafting of the manuscript. Author EMB participated in the samples and data collection. All authors also read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Brucellosis is a major zoonotic disease that is endemic in Saudi Arabia and it remains a major health problem that has not been eradicated in the country yet.
Place and Duration of Study: This retrospective study was conducted in a Saudi Hospital at Al Madinah city during the period of 1 November, 2010 to 31 October, 2011.
Methodology: All sera of patients suspected to have brucellosis (n= 65) and 18 healthy subjects were tested for brucella antibody using slide latex agglutination (SAT) and ELISA. Quantitation of IFN-ɣ was also done using ELISA.
Results: Brucellosis was detected in all age groups but the incidence was higher and reached 33.3% in age group (40- <50) years with average of 43.9±2.53 years. Male to female ratio in infected patients was 2:1 by using SAT. The incidence of seropositive cases was high (80.1%) in the three months (April, May and June), with the highest peak in May

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(46.7%). Drinking raw milk was the most encountered risk factor with a prevalence of 66.1% followed by consumption of milk products (11.9%). The most prevalent species among the examined cases was *B. melitensis* (93.3%). Among the studied cases, 60 cases (92.3%) were serologically positive for brucellosis by SAT. Among the 60 cases yielding significant titers against brucella, 14 sera (23.3%) had agglutinin levels of 1:80, 34 sera (56.7%) had titers of 1:160 and 12 sera (20%) had titers of 1:320. By estimating IgM and IgG levels in the sera of examined cases using ELISA, 52 cases (80%) had brucellaIgM while 42 cases (64.6%) had brucella IgG. Sensitivities of SAT, IgM ELISA and IgG ELISA were 91.5%, 88.1% and 71.2%, respectively compared with combined ELISA. Mean IFN-ɣ levels **±** SD in the subacute phase was 136.7±70.07pg/ml, 120.2±54.25pg/ml in the acute phase, and 121.3±51.09 pg/ml in the chronic phase of brucellosis.

Conclusion: The sensitivity and specificity of ELISA to diagnose human brucellosis was higher when combined ELISA (IgM/IgG or both) was used. Mean IFN-ɣ levels were lower, but not significantly, in the chronic phase of the disease than in the sub acute phase and healthy subjects.

Keywords: Human brucellosis; Saudi B; melitensis; B. abortus; Slide agglutination test; ELISA; IFN-ɣ.

1. INTRODUCTION

Brucellosis is one of the most common zoonotic infections worldwide, with more than 500,000 new cases reported annually [1]. The heaviest disease burden lies in the Mediterranean countries of Europe and Africa, Middle East, Central Asia, India, Mexico, Central and South America [2]. Disease incidence and prevalence rates vary widely among nations [3].

The persistent worldwide prevalence of human brucellosis causes serious public health concerns and economic loss to communities. The non-specific clinical features that overlap with a wide spectrum of other infectious and non-infectious diseases make brucellosis being labeled as the 'disease of mistakes' [4,5]. It is thus necessary to confirm brucellosis infection thorough laboratory diagnosis [6,7].

Brucellosis is endemic in Saudi Arabia [8,9], and became clearer in the early 1980s [10]. It is estimated that the annual incidence of brucellosis in Saudi Arabia is 21.4/100,000 population [11].

The Brucella species differ in degree of virulence and invasiveness. *B. melitensis* is the most invasive and produces the most severe disease while, *B. abortus* is the least invasive and causes the mildest illness [12].

Humans usually acquire brucellosis from domestic animals through direct contact or consumption of their products and are not themselves source of contagion [13]. The consumption of fresh, unpasteurized milk from camels is a traditional practice, and people believe that boiling removes the goodness from the milk [10].

Brucella species are intracellular pathogens that can survive and replicate within the mononuclear phagocytic cells of the reticuloendothelial system of the host. Following infection, a cell-mediated response is triggered [14]. Protective immunity against intracellular bacteria depends on the interplay between various T cell subsets and cytokines [15].

Cytokines appear to have an important role in the pathogenesis of brucellosis, and the Th1/Th2 balance may be involved in the susceptibility or resistance to the disease [16]. The Th1 cytokine, IFN-γ, plays an important role in activating macrophages and in limiting Brucella infections both in vitro and in vivo [17]. Therefore, it is also a well-studied cytokine in brucellosis [18,19]. Increased levels of IFN-γ in acute human brucellosis have been reported [20,21]. Circulating levels of cytokines are correlated with clinical activity in some diseases [22,23].

As human brucellosis has a serious medical impact in Saudi Arabia, the study has estimated the prevalence of brucellosis at a Saudi hospital and compared between different serological methods in diagnosis of patients with signs and symptoms of brucellosis. As the number of clinical studies on IFN-γ levels in human brucellosis is limited, serum levels of IFN-γ in patients with different disease stages were also investigated.

2. MATERIALS AND METHODS

2.1 Patients

This retrospective study was conducted on a total of 65 patients (42 males and 23 females) suspected to have brucellosis. Fever, malaise, sweating, splenomegaly, lymphadenopthy, and myalgia were the most common presenting symptoms, each with duration of less than seven days. All patients were admitted to a hospital at AlMadinah city, during the period of 1 November, 2010 to 31 October, 2011.

The study also included 18 healthy subjects (10 males & 8 females) with apparently no evidence of infection with brucellosis.

2.2 Slide Latex Agglutination

All sera of patients and control were tested for the identification of Brucella species and quantitative determination of specific brucella antibody (AB) using febrile antigen kit (Plasmatec Laboratory products, UK).

2.3 Detection of Brucellaigm / IgG by ELISA

All sera of patients and controls were tested for brucella IgMAb and brucella IgGAb using Novagnost kit (Nova Tec, Germany). The assay procedure was done with BEP® III (Fully automated instrument for running ELISA test).

2.4 Immunological Quantitative Assay of Human IFN-γ

Sera of patients and controls were tested by BEP® III ELISA using kits provided by Quantikine (R&D systems Inc., Minneapolis, USA).

2.5 Statistical Analysis

Statistical Package for Social Sciences SPSS version 13 was used. Quantitative data were presented as frequencies, percentages; arithmetic mean and standard deviation were calculated. Chi-square test (with Yates Correction) and bivariate correlation (Spearman Correlation) was used. All tests were two tailed and considered significant when $p<0.05$.

3. RESULTS

There was a significant difference among different age groups seropositive for brucellosis using SAT, IgM ELISA, IgG ELISA and combined ELISA (either IgM/IgG or both) (p=0.001, 0.000, 0.026 and 0.001, respectively).

A total of 60 brucellosis cases (92.3%) was detected by SAT (Table 1). Most of the positive cases detected by SAT (33.3%) was of age group 40- <50 years, while only 3.3% were belonging to age group 10- <20 years.

It was of interest to note that there was a significant difference in gender of brucellosis cases (p=0.010). The most prevalent species among the positive cases was *B. melitensis* (93.3%).

Table 1. Demographic characters of brucellasero-positive cases using SAT

Demographic characters	N (60)	Percentage of sero-positives	p-value
Age group (years)			
10- <20	2	3.3	
(Mean ± SD)	19.00±0.0		
20- <30	7	11.7	
(Mean ± SD)	25.6±3.00		
30- <40	14	23.3	
(Mean ± SD)	35.6±3.13		p=0.001*
40- <50	20	33.3	
(Mean ± SD)	43.9±2.53		
50- ≤60	17	28.3	
(Mean ± SD)	55.7 ±3.82		
Gender			
Male	40	66.7	p=0.010*
Female	20	33.3	
Type of Brucella			
B. melitensis	56	93.3	p=0.000*
B. abortus	4	6.7	

**p-value is significant at <0.05 level*

There was a significant difference among month of hospital admission of those positive cases by using SAT, IgM ELISA, IgG ELISA and combined ELISA (p=0.000, 0.084, 0.000, 0.000 and 0.000, respectively). From Fig. 1, it is noticed that all brucellosis cases were recovered during the period from January to June. The number of positive cases was highest in April, May and June with a monthly incidence of 21.7%, 46.7% and 11.7%, respectively. On the other hand, the examined cases during November, December, and months from July to October were negative for brucella.

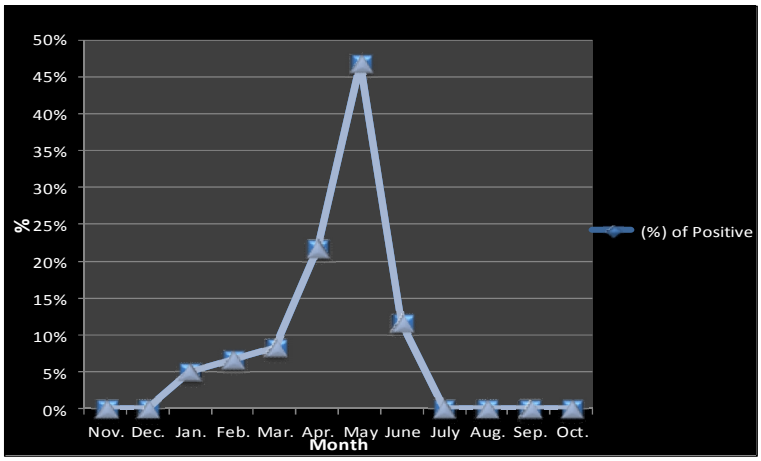

Fig. 1. The incidence of brucellosis cases in different months using SAT

There were different risk factors for infection with brucella according to combined ELISA with a high significant difference among them (p=0.000). Drinking raw milk was the most encountered risk factor for brucellosis with an incidence of 66.1% followed by consumption of milk products (11.9%). Meanwhile, animal contact, cutting raw meat and milking animals were the least encountered risk factors with an incidence of 8.5%, 8.5% and 5.1%, respectively using combined ELISA.

A high significant difference (p=0.001) was detected between percentage of positive cases and the different antibody titer. Among the 60 cases yielding significant titers against brucella, 14 sera (23.3%) had titers of 1:80, 34 sera (56.7%) had titers of 1:160 and 12 sera (20%) had titers of 1:320.

Among the suspected cases, 60 cases (92.3%) were positive by SAT, 52 cases (80%) were positive for IgM antibodies, while 42 cases (64.6%) were positive for IgG.

Mean serum IFN-γ levels in different status of brucellosis are shown in Table 2 and Figure 2. Mean IFN-γ levels appeared to be higher in healthy subjects and subacute brucellosis cases than in other groups but with no significant difference. Meanwhile, mean IFN-γ levels were lower in positive cases using combined ELISA than in other groups.

Table 2. Mean IFN-γ levels in different cases status

Status	IFN-γ (pg/ml)(Mean ± SD)
Healthy subjects	140.8±43.20
Subacute (1:80 or 1:160 by SAT)	136.7±70.07
Acute (positive cases for IgM)	120.2±54.25
Chronic (positive cases for IgG)	121.3±51.09

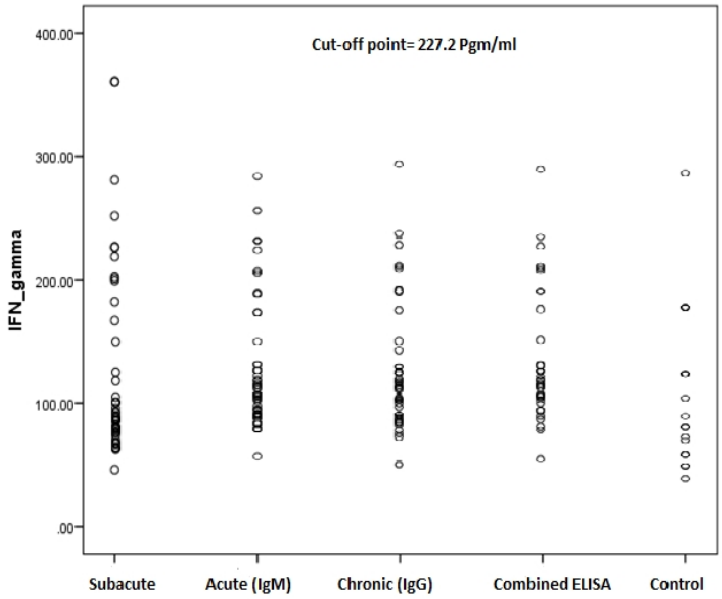

Fig. 2. Scatter diagram represents the distribution of IFN-gamma levels in subacute, acute (IgM), chronic (IgG), acute, chronic (combined ELISA) and control around cut-off value (mean control + 2 standard deviation)

4. DISCUSSION

In the present investigation, we analyzed clinical and serological characteristics of 65 suspected brucellosis cases admitted to this Saudi hospital. Sixty cases of them (92.3%) were found to be serologically positive for brucella by SAT method. Similar to our finding, Pabuccuoglue et al. [24] reported a high positivity rate of brucellosis (95.65%), while Sathyanarayan et al. [25] discovered that 30.95% of the suspected cases were having brucellosis.

Our result generally agree with a previous report of Mantur et al. [26] and De Massis et al. [27] who recorded that human brucellosis affects all age groups.

The mean age of infected patients in this study was 42.1±10.85 years with age range from 10 to ≤ 60. The age of the infected patients was 36.7± 11.69 years with range of 14-80 years [28]; 34.36±11.91 years with range of 13-78 years [29]; and 39.24±11.6 years with range of 20-76 years [30].

Age group of 40 - <50 years had the highest infection rate among our studied cases. This finding is in contrast to other studies from Saudi Arabia [27,31,32,33].

In the present investigation, 40 cases (66.6%) were males, while 20 (33.3%) were females using SAT. Similar finding, a male to female ratio of 2:1, has been reported by Al-Tawfiq & Abu Khamsin [33]. Many other studies also revealed that males were more commonly affected than females [28,31,32,34,35,36]. Mantur et al. [26] suggested that males are affected more commonly than females due to risk of occupational exposure.

In contrast to our results, Malik [37], Hussein et al. [38] and Cetinkaya et al.[39] pointed out a higher prevalence of brucellosis in females than in males. These variations in infection rate with sex are most likely related to the differences in the practice, habits and occupation between the various study populations.

The most prevalent species among our examined cases was *B. melitensis* in 93.3% of the positive cases, while *B. abortus* was detected in only 6.7% by using SAT. This result is comparable to result of Habib et al. [40] who detected *B. melitensis* in 94.45% of brucellosis cases whereas, *B. abortus* in only 3.7% and *B. suis* in 1.85%.

In Saudi Arabia, human infection with *B. melitensis* is commonly encountered (80%-100%), infection with *B. abortus* is less frequent, but infection with other species has not been reported [12,41,42,43,44].

It is thought that in brucellosis-endemic regions, the disease in humans usually peaks in June and July [27,45,46]. In the current study, there was a variation in the incidence of brucellosis from month to another. Our result is similar to that of Al-Tawfiq & Abu Khamsin 33] as the percentage of seropositive cases was high in the three months (April, May and June), with the highest peak in May, and the lowest incidence was in January. Memish & Mah [47] recorded the highest incidence of brucellosis in Saudi Arabia in spring and summer seasons.

The seasonality of brucellosis is seen in areas where local people may visit rural areas to enjoy spring and freshly expressed camel or goats' milk especially in spring and early summer [32,48,49,50].

The seasonal distribution of human brucellosis incidence is explained by the lactation period in dairy sheep and goats. Among small family-owned flocks, parturition occurs mainly from December through March and is followed in the spring and summer months by the production of milk and other dairy products (mainly soft cheese and yoghurt). The production of these homemade dairy products coincides with the peak of brucellosis incidence and continues until the end of the milking season in August and September. Parturition, abortion and lactation commonly determine the seasonality of human brucellosis, and in most climates case incidence is highest in the spring and summer months [51].

In view of transmission of brucellosis, the effect of risk factors on brucellosis incidence was evaluated. It can be concluded from our results that there were different risk factors for brucellosis but drinking raw milk was the most common risk factor similar to Al-Eissa et al. [43], Malik [37], Al-Fadhli et al. [28] and Al-Tawfiq & Abu Khams in [33].

We tried to make blood cultures for the collected samples but unfortunately the policy of the hospital prevents brucella culturing as it is highly virulent.

SAT test was used for titration of anti-brucella antibodies in the suspected brucellosis cases; and 60 cases (92.3%) were positive by SAT. It is evident that anti-brucella antibodies titers reached to 1:80 in 14 cases (23.3%), 1:160 in 34 cases (56.7%) and 1:320 in 12 cases (20%). Sathyanarayan et al. [25] found that, seven cases were positive for brucellosis and yielded significant titers against brucella, three sera (42.9%) had agglutinin levels of 1:80, three sera (42.9%) had titers of 1:160 and one sera (14.2%) had a titer of 1:320.

The highest ratio of SAT titer was 1:60 in serum of the positive cases (56.7%) in the present work. This is in accordance to Pabuccuoglue et al. [24] who reported 53 patients (57.6%) with SAT titer of 1/160, and the ratios of the other SAT titers 1/320, 1/640, and 1/280 were 23.9%, 8.7%, and 9.8%, respectively.

The prozone phenomenon may cause false- negative result, whereas, infection with cholera, tularemia and Yersinia can result in false-positive reaction in SAT [52]. Al-Dahouk et al. [53] added to the previous drawbacks of SAT, a major drawback in that it is not suitable for patient follow-up, since titers can remain high for a prolonged period.

The ELISA method has higher positivity, higher titers and the advantage of identifying different classes of antibodies in comparison to other agglutination methods. Different results may be obtained depending on the nature of anti-globulin. This situation has an effect on the sensitivity, specificity and ultimately applicability of the method [54,55]. Araj et al. [56] argued that the ELISA method should be preferred because in chronic and complicated cases, SAT and Rose Bengal tests might miss a serious portion of positive cases.

For all the above literature, we have estimated brucella specific IgM and IgG antibody levels in the sera of the examined cases using ELISA in addition to SAT. 59 cases (91.5%) of clinically suspected brucellosis yielded positive result with ELISA. Of them, 52 cases (80%) were positive for brucellaIg M, while 42 cases (64.6%) were positive for brucella IgG. Sathyanarayan et al. [25] from India, reported 30.9% (13/42) of clinically suspected brucellosis yielded positive results with ELISA. Of them, 8 cases (19%) were positive for brucellaIg M and 2 cases (4.7%) were positive for brucella IgG. While Mathai et al. [57] reported 39.1% (9/23 cases) of the clinically suspected brucellosis to be positive for IgM and 13.04% (3/23) for IgG by ELISA.

In the current study, 59 cases of clinically suspected brucellosis yielded positive result with combined ELISA. Among them, 54 cases (91.5%) were positive by SAT and yielding significant titers against brucella, 12 sera had (22.2%) agglutinin levels of 1:80,31 sera (57.4%) had titers of 1:160 and 11 sera (20.4%) had a titer of 1:320.

Sathyanarayan et al. [25] detected 17/42 samples (40.47%) of clinically suspected cases exhibited agglutinins against brucella. Taking into consideration the results of ELISA and agglutination tests together, 45.23% of sera samples showed the presence of agglutinins against brucella, and 30.9% of their clinically suspected brucellosis, yielded positive results with ELISA.

Mantur and his associates [58] explained that during the first week of infection, IgM antibodies against lipopolysaccharide antigens appear in the serum, followed by IgG antibodies as early as the second week. Both antibody isotypes peak during the fourth week. They also added that brucella specific IgM and IgG are still the most common and useful measures for the laboratory diagnosis of brucellosis as they are faster and reduce risk of laboratory acquired infections due to handling brucella culture.

The diagnostic efficacy of SAT & ELISA in the diagnosis of suspected brucellosis cases in this investigation was compared. We have assumed IgM and IgG ELISA tests, when used in conjunction, as the gold standard by which the other tests were compared according to Sathyanarayan et al. [25]. Sensitivity (%) of SAT, IgM, IgG compared to combined ELISA was 91.5, 88.1, 71.2; Specificity (%) was zero, 100,100; Positive predictive value was 90,

100,100; Negative predictive value was zero, 46.2, 26.1, respectively (Data not shown in a table).

In view of other studies, sensitivity of SAT, IgM ELISA and IgG ELISA were 95.6%, 79% and 45.6%, respectively by Memish et al. [59]; 83.7%, 61.9%, and 49.5% by Sirmatel et al. [60]; and 94.3%, 97.1% and 71.4% by Ciftci et al. [61].

Performance in ELISA tests varied in different studies. Sisirak & Hukic [62] reported low sensitivities, 64.8% for IgM ELISA and 56.1% for IgG ELISA. However, Araj et al. [56] reported very high sensitivities for ELISA tests of 91 and 100% for IgG ELISA and IgM ELISA, respectively.

In comparison, Sathyanarayan et al. [25] in the diagnosis of human brucellosis in cases of pyrexia of unknown origin detected sensitivity (%) of blood culture, slide agglutination and ELISA IgM & IgG (in conjunction) was zero, 38.46. 100; specificity (%) was 69.04, 100, 100; positive predictive value was zero, 100,100; negative predictive value was 69.04, 78.37, 100, respectively.

Recently, the awareness increased to the whole-blood IFN-γ assay as a quantitative in vitro assay for a direct read-out of Ag-specific cell-mediated immune responses to infectious diseases. Riber et al. [63] confirmed that the IFN-γ assay is robust in severe intracellular infections like brucella.

By evaluation levels of IFN-γ in sera of examined cases using ELISA, the mean IFN-γ levels in the positive cases of brucellsis was lower than the healthy subjects. But on comparing the mean IFN-γ in different disease status and controls, a higher mean IFN-γ levels but with no significant difference was detected in the subacute brucellosis cases than in other groups.

Reports on the role of IFN-γ immunological activity in acute human brucellsis were controversial. Similar to our result, Al Ali et al. [64] reported a significant decrease of IFN-γ in all disease stages. The mean serum IFN-γ levels (pg/ml) of controls, antibody titer 1/80, 1/160, 1/320 were 99.4±13.6, 22.5±26.4, 13.3±11.8, 6.6±7.5, respectively. Also, Rodriguez-Zapata et al. [19] concluded that phytohaemagglutinin-stimulated T-lymphocytes from untreated patients with acute brucellosis have defective IFN-γ production.

In contrast to our finding, significantly higher levels of IFN-γ were found in the serum of patients with brucellosis compared with patients without brucellosis and controls [65]. Moreover, Giambartolomei et al. [21] and Demirdag et al. [66] reported increased levels of IFN-γ in acute human brucellosis than controls. Ahmed et al. [20] reported significant rise of the levels of IL-8, IL-12 and IFN-γ in the serum of patients with brucellosis compared with patients without brucellosis and controls.

5. CONCLUSION

We can conclude that lifestyles and the consumption of fresh dairy products played a significant role in the high incidence of brucellosis in that hospital in Almandine Region.

ELISA was more sensitive and specific compared to the agglutination test. Further, the IgM and IgG ELISA tests, when used in conjunction were more reliable test for the diagnosis of brucellosis. This is the same result of Sathyanarayan et al. [25].

Due to the high prevalence rate of brucella in this study, we recommend a mandatory laboratory based surveillance of brucella be incorporated into the surveillance system especially during the expected infection months. The isolation, identification and molecular characterization of *Brucella* spp. in human and the different livestock species needs to be undertaken to identify the source of infection and design appropriate control measures.

Finally, the results indicated the importance of studying the cytokines in relation to the antibody titers. However, the study of certain cytokines as IL-10, IL-2, IL-4, IL-8, IL-12, TNF-α and transforming growth factor-β appear crucial for further study to understand the immunological responses at different stages of the disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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