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ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Study of some cytokines in patients with brucellosis in Najaf province

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ABSTRACT

Background: To evaluate the levels of pro-inflammatory cytokines for 'Th1/Th2 brucellosis' patients serum to determine 'antimicrobial susceptibilities' of *Brucella* strains.

Methodology: The study comprised 35 individuals with acute brucellosis, and 21 patients with chronic brucellosis, identified clinically. A group of 20 healthy adult persons was also enrolled in the study. For all of the participants, blood cultures and standard tube agglutination tests for *Brucella* were performed. ELISA was used to detect cytokine levels in serum samples collected following the treatment period and detect *Brucella* strains celebrated from different sites of infection, and in order to find-out the susceptibilities against antibiotics including rifampicin (RIF), streptomycin (STR), doxycycline (DOX), and ciprofloxacin (CIP) via the gradient strip (E test).

Results: The patients included 22 (39.29 percent) males and 34 (60.71 percent) females based on their gender and age. Fever was the most common clinical feature (92%), sweating came in second place (75%), followed by back pain with 18%. Patients with brucellosis had substantially higher blood levels of interleukin-6 (IL-6), interferon (IFN), and tumor necrosis factor-alpha (TNF-alpha) analogous to the control group (P = 0.05). IL-4 and IL-2 levels between the sick and control groups did not vary significantly. The amounts of these cytokines were found to be identical in acute and chronic individuals. Rendering to "minimum inhibitory concentrations (MIC) 90 levels," CIP was determined to be the most effective antibiotic. Then followed DOX and then STR, correspondingly. All of the samples were CIP and DOX susceptible; 18 (23.2%) strains were discovered to be only mildly sensitive to RIF, with MIC50 and MIC90 maximal values.

Conclusions: Our findings point to an important role for IFN and proinflammatory cytokines in brucellosis pathogenesis. However, these changes were shown to be unrelated to the severity or activity of the illness. Furthermore, all strains were identified as *Brucella* strains. ciprofloxacin, doxycycline, rifampicin and streptomycin were found to be effective for the treatment of brucellosis.

Key words: Antimicrobial Susceptibilities; Brucellosis; Cytokines; Interferon; Pro-Inflammatory Cytokines

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1. INTRODUCTION

Brucellosis is a widespread infection. This multi-systemic disease is common in both humans and animals, and it is spread between infected animals either directly or indirectly. It is also called Malta fever, intermittent fever, Mediterranean fever, as well as undulant fever in humans. Brucella is a non-capsular, Gram-negative, non-motile, intracellular bacterium capable of causing chronic zoonotic infections in people. Brucellosis affects the liver, spleens, and kidneys.[1] It also affects the blood vessels, heart, digestive system, skin, musculoskeletal system, eyes, bone marrow and nervous system. The disease varies from slight flu like symptoms to systemic infections with severe complications. The response of immune system to Brucella spp. has the spectrum from innate to adaptive immunity.[2]

Brucellosis in human is a worldwide public health problem that leads to sterility, pregnancy failures and economic burden, chiefly in regions known to harbor repeated infections to animals.[1] Brucella's intracellular existence plus its definite lipopolysaccharide (LPS) component remain significant parts used to keep cover. Brucella is identical to Gram negative bacteria which produce toll-like receptors -TLR2, TLR4, as well as TLR5; however, these are greatly reduced.[3]

The convalescent patients have percentages of Type 1 T-helper (Th1) cells greater than healthy controls, and it mostly shows important role in the resistance of host to pathogens specially for intracellular infections by bacteria. The cytokines express from T lymphocytes altered in person for diverse periods. In these patients the rate of CD4+ T lymphocytes in addition the proportion of CD4/CD8 cells were essentially lower than individuals of healthy controls.[4]

Th1 cells mediate the effector pathways required for intracellular pathogen resistance in addition to the creation of the interferon gamma (IFN-γ), which remains an essential regulator of brucellosis. However, patients with brucellosis in chronic state have reduced quantity of Th1 lymphocytes throughout, matched with patients with brucellosis in acute state.[3]

The first step of protection in body against Brucella consists of phagocytic action by many cells as polymorphonuclear (PMN) cells, macrophages, natural killer (NK) cells, complement system, dendritic cells, and chemokines. The definite immune response produced through Brucella infection includes three chief mechanisms. The secretion of interferon is done first by CD8+ T cell, CD4+ T cell, and γδ T cell, which trigger the task of macrophages as bactericidal tools and inhibits Brucella intracellular persistence.[5] The special influences of Brucella on cells that present antigen still fundamentally effect the response of adaptive immunity to develop to the bacteria.[3] The cytotoxic effect is the second by CD8+ T cells, that could eradicate macrophages harboring bacteria; finally, the subtypes of Th1 antibody, as IgG2a also IgG3, supports phagocyte action. Additionally, cytokines remain important in producing both specific as well as non-specific immune responses.[5]

Although the Th-2 action of cytokine, as IFN-γ, IL-2, besides IL-4, IFN-γ and IL-2 are formed from both CD4+ and CD8+ T cells, cytokines have many actions including development of defense cells and their maturation, differentiation, then activation. Such as, IL-4 (Th2 cytokines) prompts IgG1 antibody development by differentiation of the naive CD4+ T cells to Th2 cells, while IFN-γ that was Th1 cytokines prompts IgG2 antibody development through differentiation of the naive CD4+ T cells to Th1 cells.[6]

The effectiveness of the treatment is mostly determined by type of antibiotic use and disease-treating strategy. Submitting to the WHO, the brucellosis treatment formula involves a mixture of doxycycline 100 mg twice a day, besides rifampicin 600 mg/day. Both of these are administered for 6 months and streptomycin 1 g/day IM, is used for 21 days.[7] However, not enough data is available about the antibiotic resistance of Brucella strains disseminating in Najaf (Iraq), and such research may help to increase treatment effectiveness.

The goal of the current research was to look at levels of IL-6, TNF-α, TGF-1, Th1 (interferon-γ, IL-2) besides Th-2 (IL-4) cytokines found in acute as well as chronic brucellosis. Additionally, we studied how they change with treatment, and study of the antibiotic susceptibility in *Brucella* strain sequesters from 5 regularly taken antibiotics.

2. METHODOLOGY

2.1. Characterization of *Brucella* sp.

The study included patients, ages 10-50 y, in various hospitals in Al-Najaf province. Written signed permission was obtained from every patient or his/her next of kin to include in the study. The research was approved by the institutional ethics board of the University of Kufa (2754/2012/1/29). The study was accomplished under Iraqi and foreign ethics and privacy rules according to the guidelines of the Declaration of Helsinki, The Belmont Report, CIOMS Guideline, and International Conference on Harmonization of Good Clinical Practice; our IRB adheres to the International Guideline for Human Research Safety (ICH-GCP).

The diagnostic criteria was: (a) isolation of just a *Brucella* species from blood cultures; and (b) detection of a 1/160 antibody levels to *Brucella* using the standard tube agglutination (STA) procedure in the presence of a suitable clinical presentation, such as intense or pernicious onset of malaise, fever, anorexia, weight loss, arthralgia, headache, sweating, and back pain, or clinically. Hemoglobin, CRP, and WBC count reflect some of the physical besides laboratory characteristics that were measured.

2.2. ELISA assay for cytokine estimation

The following cytokines were calculated from serum samples taken from the patients: TGF-β1 and interleukin (TNF-α, IL-6, IL2 and IL-4), using ELISA kits (Abcam, USA). Acute and chronic patients for brucellosis identified clinically were included in the study with pre- and post-treatment.

2.3. Determination of minimum inhibitory concentrations (MICs)

A method evaluated antimicrobial resistance by using the E test technique (Biomerieux®, France), the MIC for rifampicin (RIF), streptomycin (STR), doxycycline (DOX), and ciprofloxacin (CIP) were identified. Each *Brucella* strain's inoculum was produced in Muller-Hinton broth (Oxoid), 0.5 McFarland turbidity, and added 5% sheep blood before being swabbed onto Muller-Hinton agar plates. The plate containing the E test strips was incubated for 48 h at 37 °C. [9] The strategies for Clinical plus Laboratory Standards (CLSI) reference ranges aimed at DOX as well as STR used for *Brucella* species, and slow-growing bacteria's (Haemophilus) used as standard values for CIP and RIF were used to evaluate the determination of MIC.[10] For every stage in this research, *B. melitensis biovar* (bv) 1(16 M), *B. melitensis* bv 3(Ether), *B. abortus* bv 1 (NCTC10093), *B. abortus* bv 3(Tulya), *B. suis* bv 1(1330), *E. coli* ATCC 25922 plus *S. aureus* ATCC29213 were utilized conformance. The MIC comprises "the lowermost antibiotic concentration which stops bacterial growth". Moreover, it was accepted to inhibit 50% for them as MIC50 besides 90% for them as MIC90.

2.4. Statistical analysis

Statistical analysis was carried out by using Student's *t* test. Variables were estimated as Mean ± SE. The analysis of variance (ANOVA) was used.

3. RESULTS

A total of 76 persons took part in this study, comprising 56 brucellosis patients besides 20 healthy people. A gender plus age distribution of patients is shown in Table 1. The patients were 22 (39.29 %) male and 34 (60.71 %) female. Patients varied in age from 33 to 37 y old, with larger numbers in some age categories; 23-27 y old, with 22 (39.28 %) and 13 (23.2 %) patients correspondingly.

Table 1: The age and gender distribution of brucellosis patients

Age	Male	Female	No. of patients
18-22	1	3	4 (7.14%)
23-27	5	8	13 (23.2%)
28-32	4	7	11(19.6%)
33-37	10	12	22 (39.28%)
38-42	2	4	6 (10.7%)
Total	22 (39.29%)	34 (60.71%)	56 (100%)

Table 2: Clinical characteristics of patients with brucellosis

Clinical characteristics	Percentage
Malaise	71%
Weight loss	52%
Lack of appetite (anorexia)	45%
Fever	92%
Sweating	75%
Arthralgia	70%
Headache	50%
Back pain	18%

Table 3: Physical and laboratory findings of patients with brucellosis

Parameters	Mean ± SD
ESR (mm/h)	39.3 ± 1.4
Hemoglobin (g/dL)	13.4 ± 0.44
White blood cell count (cell/mm3)	5736 ± 23
C-reactive protein (g/dL)	6736.2 ± 14.22

Table 2 shows that the fever was the most common clinical feature (92%), sweating was second with 75%, and back pain at 18%.

Table 3 shows the physical and laboratory results of brucellosis patients. Mean Hb levels in patients were 13.4 ± 0.44 g/dl, while the mean ESR was 39.3 ± 1.4 mm/h.

Table 4 shows the mean serum cytokine levels of patients post-treatment, acute patients, chronic patients, and healthy controls. TNF-α, TGF-1, and IL-6 mean blood levels (32.81 ± 1.13, 489.5 ± 1.04, 466.4 ± 5.18 and 9.10 ± 0.61 pg/mL, were higher of post-

Table 4: Cytokine levels in patients are compared to those in healthy group

Cytokines	BD groups (n = 56)		Post-treatment	Healthy group (n = 20)
	Acute (n = 35)	Chronic (n = 21)		
TGF-β1	442.1 ± 0.99	469.3 ± 1.93	489.5 ± 1.04	466.4 ± 5.18
IL-6	16.38 ± 2.01	20.96 ± 0.18	9.10 ± 0.61	2.1 ± 0.15
IL-4	1.43 ± 1.75	3.1 ± 0.17	2.46 ± 1.92	0.72 ± 1.42
TNF-alpha	49.48 ± 1.45	49.87 ± 7.53	32.81 ± 1.13	10.46 ± 1.2
IL-2	0.94 ± 0.41	0.42 ± 0.28	1.82 ± 0.64	1.36 ± 0.85

P < 0.05; Data presented as Mean ± SE

treatment in definite patients related to the control group (mean ± SD, 10.46 ± 1.2 and 2.1 ± 0.15 pg/mL, (P < 0.05). However, there were no significant differences in the levels of IL-4 and IL-2levels between the patient post-treatment with control groups (P < 0.05), which were 2.46 ± 1.92 and 1.82 ± 0.64 respectively; P < 0.05).

Furthermore, there were no changes between chronic and acute brucellosis patients. Table 4 shows the cytokine levels in two categories of patients with infections, as well as the control group.

According to the findings of the E test, DOX and CIP were effective against all strains, 54 (96.4%) were STR-sensitive and 54 (76.7%) were RIF-sensitive (Table 5).

CIP, DOX, and STR were the most successful agents used for strains of *Brucella*, according to MIC90 values. RIF yielded the greatest MIC50 and MIC90 values. Table 3 lists the MIC varieties, MIC50 values, besides MIC90 values of the numerous antimicrobial drugs utilized against isolates in this research.

4. DISCUSSION

In acute and chronic brucellosis patients, mean blood levels for IL-6 besides TNF-α were greater than for healthy group (P < 0.05).

While, the Th1 role, in addition to formation of IFN-γ, remains vital to regulate this bacterium, however, the bacterial growth and distribution remains limited through IFN-γ,

TNF-α, IL-17, and the restricted surroundings oxygen. A core granuloma is filled with them.[3]

According to Lin et al., it was found that higher levels of IL-4, IL-10, IL-17, IL-6, IFN-γ, and TNF-α existed in those having brucellosis than in controls (P < 0.05).[10] in spite of this, only IL-6 plus INF-γ levels are independent factors related with the seriousness of brucellosis. Because IL-6 is so important in inflammation, it's possible that it plays a role in the brucellosis process and pathogenesis.

TNF-α was found to be associated with an increase in IFN-γ levels. In addition to high levels in the acute phase of the disease, chronic disease has also been shown to have high levels. B. abortus has been shown to induce a Th1 response both in vivo and in vitro.[11]

In a small number of studies including acute brucellosis patients, IL1 and IL4 levels were undetectable in the blood, but IL2 and IFN-Q were "importantly greater for brucellosis patients" than in the control group.[5,6] In addition to research suggesting that brucellosis triggers a Th1 immune response, Guimares et al. and Callaghan discovered that throughout severe phase in infection, IL-4 levels in serum surged in children with brucellosis.[11,12]

Increased production of these lead to a worsening of the disease's course, as well as a loss of Brucella immunity as a result of an overly aggressive Th2 response. While serum IFN-Q levels were significantly greater in acute

Table 5: Antibiotic susceptibility of Brucella strains (μg/ml)

Antibiotics	No.	Sensitive	Intermediate	Resistant
RIF	56	43 (76.7)	13 (23.2)	–
DOX	56	56 (100)	–	–
CIP	56	56 (100)	–	–
STR	56	54 (96.4)	2 (3.6)	–

Data presented as n (%)

Table 6: MIC ranges and (MIC50 and MIC90) standards of the numerous antimicrobial agents against Brucella species

Antibiotics	No.	MIC range	MIC50	MIC90
RIF	56	0.01–2	1.2	1.7
DOX	56	0.02-1.2	0.05	0.22
CIP	56	0.005-0.3	0.082	0.134
STR	56	0.07-0.8	0.21	0.6

Data presented as μg/ml

brucellosis patients in our study, IL4 levels were significantly lower and did not differ significantly from the control group.

The CLSI has not developed an identical antimicrobial susceptibility assessment designed for Brucella species. According to CLSI guidelines, in our research drug susceptibilities were estimated. The E test system was utilized, that is less labor-intensive and time-consuming than other tests while still being reliable, reproducible, and practical.[8]

The MIC90 values of DOX against Brucella species were reported to be 0.25 g/ml in numerous studies conducted in various countries.[13] On the other hand, this figure for MIC90: 32 was deemed to be fairly high in a study in China.[14] Based on MIC readings, Etiz et al. stated that DOX was less efficient than trimethoprim/sulfamethoxazole (TMP/SXT),[8] in contrast to these investigations. According to the MIC90 value in our investigation, DOX was the second most effective drug against Brucella strains after CIP.

A study by Deshmukh in Qatar revealed a greater resistance level of RIF 48%.[13] In Egypt, according to Abdel-study Maxoud's, 19% of the strains may have RIF resistance.[15] Turkey has between 2.1 and 75% of Brucella strains had intermediate resistance to RIF.[8] In our investigation 20.7% of the Brucella strains exhibited moderate RIF resistance. Due to its good oral absorption and the fact that it reaches large quantities in phagocytic cells, CIP is a significant option in the treatment of brucellosis.[13]

The MIC ranges has been shown to be 0.064-8 g/ml in several investigations, and are still within the sensitivity range.[8,13,15] The MIC range in our study was 0.07-0.8 g/ml, which was consistent with earlier research.

5. CONCLUSION

We conclude that IFN besides other proinflammatory cytokines, shows a crucial action in infection of brucellosis. In spite of some contradictory findings in the literature, it appears that Brucella infections trigger the Th1 but not the Th2 response. However, further clinical research is required in this area. Also, the study demonstrates 100% of Brucella strains in humans of Najaf/Iraq were sensitive to doxycycline and ciprofloxacin. This finding might be used to improve treatment regimens.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

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9. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

10. Authors' contribution

ZYK Evaluated, and edited the manuscript. Other authors helped in conducting the study, in literature search and preparation of the manuscript. All authors have read and approved the final draft of the manuscript.

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