ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Association of anti-brucella antibodies with circulatory IL-17, IL-18 and INF-γ in patients with brucellosis in Diyala province-Iraq

[1]Shahad Kh. Al-Qaisi, [2]Hasan, A. SH., [3]Maha F. Nazzal
[1]Middle Technical University, [2]Baquba Technical Institute,
[3]Iraq College of Medicine, University of Diyala, Iraq
Department of Biology, College of Education for Pure Science, University of Diyala, Iraq
shahadalqaisi2019@gmail.com

Abstract:
Background: Brucellosis, caused by the intracellular pathogens brucellae, is one of the most prevalent bacterial zoonosis of worldwide distribution. The disease is highly prevalent in East Mediterranean region. Cytokines are key players in protection against brucellosis, mediating both innate and adaptive immune responses.
Objective: The aims of this study are exploration the association of anti-brucella IgM and IgG antibody with the detection of circulatory IL-17, IL-18 and INF-γ.
Patients and method: A total of 55 patients who were clinically suspected as having brucellosis were included as patients group and 35 apparently healthy individuals as control group. The highest age range was 20-29 years. The mean ± SD and the age range of the patients and control groups were 29.8 ± 10.1 (15-59) and 33.8 ± 11.0 (18-58) years respectively. 20% of patients were males and 80% were females, while, 37.1 % of controls were males and 62.9% were females. The study groups were allocated from Baquba Teaching Hospital and some Healthcare Centers. A questionnaire form was preconstructed including socio-demographic and clinical information. Blood samples were collected from both study groups, sera were separated and submitted for slide agglutination (Rose Benal) test (Spin-React, Spain), Anti-Brucella IgM and IgG (Demeditec - Germany) as well as for detection of serum Interleukin-17, Interleukin-18, and Interferon –γ (My biosource -USA). Human privacy was respected by obtaining verbal consent from all participants. Statistical analysis was done using SPSS version 25 and p values ≤ 0.05 were considered significant.
Results: primarily, the clinical signs are highly associated in brucellosis patients. Moreover, consumption of milk and meat, intrafamilial clustering of cases and disease history are associated with brucellosis. The results found that 18 (32.7%) of the patients and 2 (5.7%) of the control were positive for anti-brucella IgM with statistically significant difference (P= 0.003). The anti-brucella IgG positivity rate among patients was 31(56.4%) while that of the control group was 4 (11.4%), with statistically significant difference (P= 0.0001). The IL-17 positivity rate among brucella patients was 32(58.2%) versus 15 (42.9%) among controls, with statistically insignificant difference (P= 0.156). the IL-18 positivity rate among patients was 27(49.1%) which is insignificantly higher than that of controls 11(31.4%), (P= 0.098). The positivity rate of **INF-γ** in patient group was 52(94.5%) which is highly significant compared to controls 12(34.3%), (P= 0.0001). The IL-17 and INF-γ were higher among IgM positive patients while the IL-18 was significantly higher among patients with IgG positivity.
Conclusion: Patients with acute brucellosis had higher Iinterleukin-17, and interferon-γ positivity, while the IL-18 was highly associated in patients with persistent brucellosis.

Keywords: Brucellosis, Interleukin -17, Interleukin-18, Interferon -γ

Introduction:

Brucellosis is a zoonotic and contagious bacterial infectious disease caused by the genus brucella. There are 10 species of brucella that have been classified on the basis of primary host specificity, biochemical characteristics, and antigenic component. B. melitensis and B. abortus as the two most common brucellae that cause human infection in East Mediterranean region (Elfaki et al., 2005). Brucella has the propensity to localize inside macrophages of the liver, spleen, bone marrow, uterus, heart, and brain with protean clinical manifestations (Mantur et al., 2006). The successful intracellular stealthy lifestyle has evolved through multiple strategies to evade immune response mechanisms, obligate the host cells to form

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

a microenvironment conducive to its survival, reproduction and survive in the host cells for a long time, which eventually leads to the formation of chronic persistent infection (Ahmed et al., 2016; Jiao et al., 2021). The intra- macrophages existence of brucellae represents one of the strategies for consequent alterations in both innate and adaptive immune responses (Elfaki et al., 2015).

Cytokines are key players in protection against brucellosis, mediating both innate and adaptive immune responses. IL-12 produced by B cells and macrophages leads to a Th1 response and induction of interferon-γ, which activates macrophages (de Figueiredo et al., 2015). The early IL-10 production by CD25+ CD4+ T cells modulates macrophage function and contributes to an initial balance between pro-inflammatory and anti-inflammatory cytokines that is beneficial to the pathogen, thereby promoting bacterial survival and persistent infection (Xavier et al., 2013). Experimentally, the levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-12, and tumor necrosis factor alpha (TNF-α) were elevated in B. abortus infections, especially for IFN- γ, with greater pathological changes in the liver, while the IL-10 level was lower and the levels of IL-1β, IL-4, IL-5, and IL-6 were slightly changed (Wenpeng et al., 2013). The key function played by IFN-γ-producing Th1 CD4+ T cells in the control of B. melitensis infection, whereas IFN-γ-producing CD8+ T cells or B cell-mediated humoral immunity plays only a modest role in the clearance of bacteria during primary infection. In the presence of a Th1 response, Th2 or Th17 responses do not really develop or play a positive or negative role during the course of B. melitensis infection (Vitry et al., 2012). On the other hand, it has been demonstrate that IL-10 modulates the proinflammatory immune response to B. abortus infection leads to bacterial clearance which was preceded by an enhanced IFN-γ, TNF-α and IL-17 responses in both the serum and the spleen. Additionally, dendritic cells from produced elevated levels of IL-12 and TNF-α (Corsetti et al., 2013).

The Th17 cells, a distinct subset of CD4+ T cells, produce various cytokines, namely IL-17, IL-6, IL-9, IL-21, IL-22, IL-23, IL-26, GM-CSF, MIP-2, monocyte chemoattractant protein-1 and TNF-α. The IL-17 family, is an important inflammatory cytokine, playing principal roles in both innate and adaptive immunities (Bedoya et al., 2013; Isailovic et al., 2015). The role of IL-17 was differentially relevant with respect to brucella species. IL-17-producing cells, found in the lamina propria, play an essential role against microorganisms infecting the gastrointestinal tract. Its production has also been observed in the lung and oral cavity mucosa (Marks and Craft, 2009). IL-17 is among critical determinants of risk, severity or protection of infectious diseases (Park et al., 2005). As the balance between Th1 and Th2 cytokines can cause resistance or susceptibility to infection with brucella species, Th1 cytokines inducing resistance, whereas Th2 cytokines cause predisposition to brucellosis (Gaffen, 2008; Romagnani, 2008). Furthermore, the role that IL-17 plays in the protection against brucellosis induced by vaccination in the intestinal mucosa (Pasquevich et al., 2011).

The IL-18 is produced mainly by macrophages. It is a proinflammatory cytokine that facilitates type 1 responses. Together with IL-12, it induces cell-mediated immunity following infection. Together with IL12 acts on CD4, CD8 T cells and NK cells to induce IFN-γ production (Yasuda et al., 2019). Experimentally, it has been found that combined inoculation of IL-12 and IL-18 reduced the number of B. abortus in the spleen and that the effect of the treatment was mediated by an increased capability of spleen cells to produce INF- γ at the early phase of infection (Pasquali et al., 2002). Conversely, once the infection was established, B. abortus selectively limits IL-18 secretion without affecting endogenous IFN- γ production (Fernandez-Lago et al., 2005).

Patients and Method:

A total of 55 patients who were clinically suspected as having brucellosis and were tested positive for agglutination (Rose Bengal) test were included as patients group and 35 apparently healthy individuals were included as control group. In both patient and control groups, the highest age range was 20-29 years. The mean ± SD and the age range of the patients and control groups were 29.8 ± 10.1 (15-59) and 33.8 ± 11.0 (18-58) years respectively. 20% of patients were males and 80% were females, while, 37.1 % of controls were males and 62.9% were females. The study groups were allocated from Baquba Teaching Hospital and some Healthcare Centers. A questionnaire form was preconstructed including socio-demographic and clinical information. Blood samples were collected from both study groups, sera were separated and submitted for slide agglutination (Rose Benal) test (Spin-React, Spain), Anti-Brucella IgM and IgG (Demeditec - Germany) as well as for detection of serum Interleukin-17, Interleukin-18, and Interferon –γ (My biosource -USA). Human privacy was respected by obtaining verbal consent from all

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

participants. Statistical analysis was done using SPSS version 25 and p values ≤ 0.05 were considered significant.

Results:

In both patient and control groups, the highest age range was 20-29 years, 45.5% and 31.4% respectively. However, the difference between the two groups was statistically insignificant (P= 0.162). The mean ± SD and the age range of the patients and control groups were 29.8±10.1 (15-59) and 33.8±11.0 (18-58) years respectively, with insignificant difference statistically (P= 0.078), as shown in table (1).

Table (1): Distribution of age categories in patients and control groups.

Age (Ys)	Patients		Control		P value
	No.	%	No.	%	
< 20 years	6	10.9	2	5.7	0.162 *
20---29	25	45.5	11	31.4	
30---39	17	30.9	10	28.6	
40---49	3	5.5	6	17.1	
≥ 50 years	4	7.3	6	17.1	
Total	55	100%	35	100%	
Mean ± SD (Range)	29.8 ±10.1 (15-59)		33.8 ±11.0 (18-58)		0.078 **

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

**Significant difference between two independent means using Students-t-test at 0.05 level.

Table (2) showed that within the patients group, 44 (80%) were female and 11 (20%) were male. While in control, 22 (62.9%) were female and 13 (37.1%) were male. The difference between the two groups was statistically insignificant (P= 0.073).

Table (2): Distribution of gender in patients and control groups.

Gender	Patients		Control		P value
	No.	%	No.	%	
Male	11	20.0	13	37.1	0.073
Female	44	80.0	22	62.9	
Total	55	100	35	100	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

Regarding the residence, the results found that there was statistically insignificant difference between the patients and control groups (P= 0.809). The house breeding of productive animals showed that patients had higher rate of house breeding compared to control, but the difference was failed to reach the levels of statistical significance (P = 0.099). Concerning the consumption of dairy products and meat, there was a statistically significant difference between patients and control (P= 0.0001). The intra-familial transmission of brucellosis other than the index case showed that patient group had higher rate of intra-familial clustering compared to control (P= 0.045). About the disease history, the recent cases among patients was significantly higher than that of the control (P= 0.0001), as revealed in table (3).

Table (3): study groups variables.

Variables		Patients		Control		P value
		No.	%	No.	%	
Residence	City	30	54.5	20	57.1	0.809
	Village	25	45.5	15	42.9	
Home animals	Yes	20	36.4	7	20.0	0.099
	No	35	63.6	28	80.0	
Consumption of milk and meat	Yes	50	90.9	21	60.0	0.0001*
	No	5	9.1	14	40.0	
Intra-familial brucellosis	Yes	24	43.6	8	22.9	0.045*
	No	31	56.4	27	77.1	
Disease history	No infection	-	-	34	97.1	0.0001*
	New infection	44	80.0	1	2.9	

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

	Old infection	11	20.0	-	-	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

Results in table (4) showed that the patients had significantly higher rate of fever (89.1% Vs 11.4), (P= 0.0001). Night sweat (80.0% Vs 6.7%), (P= 0.0001). Likewise, the patients had significantly higher rate of joint pain as compared to control (85.5% Vs 40%), (P= 0.0001).

Table (4): Clinical variables among study groups.

Variables		Patients		Control		P value
		No.	%	No.	%	
Fever	Yes	49	89.1	4	11.4	0.0001*
	No	6	10.9	31	88.6	
Night sweat	Yes	44	80.0	2	5.7	0.0001*
	No	11	20.0	33	94.3	
Joint pain	Yes	47	85.5	14	40.0	0.0001*
	No	8	14.5	21	60.0	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

The results of Rose Bengal test showed that all patients were positive for Rose Bengal (100%), while, only 2(5.7%) of the control were positive with a statistically significant difference (P= 0.0001), table (5).**Table (5): Results of Rose Bengal test among study groups.**

Test		Patients		Control		P value
		No.	%	No.	%	
Rose Bengal	Positive	55	100.0	2	5.7	0.0001*
	Negative	-	-	33	94.3	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

In table (6), 18 (32.7%) of the patients and 2 (5.7%) of the control were positive for anti-brucella IgM with statistically significant difference (P= 0.003). The results found that the anti-brucella IgG positivity rate among patients was 31(56.4%) while that of the control group was 4 (11.4%). the difference was statistically significant (P= 0.0001),

Table (6): Distribution of anti- IgM positivity rate among study groups.

Serological Marker		Patients		Control		P value
		No.	%	No.	%	
Anti-IgM	Positive	18	32.7	2	5.7	0.003*
	Negative	37	67.3	33	94.3	
Anti-IgG	Positive	31	56.4	4	11.4	0.0001*
	Negative	24	43.6	31	88.6	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

The IL-17 positivity rate among patients was 32(58.2%) versus s 15 (42.9%)of the controls with a statistically insignificant difference (P= 0.156). The IL-18 positivity rate among patients was 27(49.1%) which is higher than that of controls 11(31.4%). However, the difference was failed to reach the level of statistical significant (P= 0.098). The positivity rate of **INF-γ** in patient group was 52(94.5%) which is highly significant compared to that of control group 12(34.3%), (P= 0.0001) as shown in Table (7).

Table (7): Distribution of Interleukins positivity rate among study groups.

Interleukins Marker		Patients		Control		P value
		No.	%	No.	%	
IL-17	Positive	32	58.2	15	42.9	0.156
	Negative	23	41.8	20	57.1	
IL-18	Positive	27	49.1	11	31.4	0.098
	Negative	28	50.9	24	68.6	
INF-γ	Positive	52	94.5	12	34.3	0.0001*

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

	Negative	3	5.5	23	65.7	

*Insignificant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level

Concerning the IL-17, the results found that 12(37.5%) who were anti-brucella IgM positive were also positive for IL-17 against 6(26.1%) who were positive for IgM but negative for IL-17. The difference was statistically insignificant (P= 0.374). Furthermore, 6(22.2%) who were positive for anti-brucella IgM were also positive for IL-18 versus 12 (42.9%) who were positive for IgM but negative for IL-18. The difference was statistically insignificant (P= 0.103). Lastly, all 18(34.6%) of those positive for anti-brucella IgM were positive for INF-γ versus 34 (65.4%) who were positive for INF-γ but negative for IgM. The difference was statistically insignificant (P= 0.214), table (8).

Table (8): Association of anti-brucella IgM with cytokine variables.

Cytokines variables		Anti-brucella IgM				P value
		Positive		Negative		
		No	%	No	%	
IL-17	Positive	12	37.5	20	62.5	0.374
	Negative	6	26.1	17	73.9	
IL-18	Positive	6	22.2	21	77.8	0.103
	Negative	12	42.9	16	57.1	
INF-γ	Positive	18	34.6	34	65.4	0.214
	Negative	-	-	3	100.0	

*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

The results in table (9) found that 21(65.6%) who were positive for IL-17 were also positive for IgG against 10(43.5%) who were positive for IgG but negative for IL-17. The difference was insignificant (P= 0.102). The results also found that 12(44.4%) of those positive for IL-18 were also positive for IgG, while 19(67.9%) were positive for IgG but negative for IL-18. The negativity rate was higher but the difference was failed to reach the level of statistical significance (P=0.080). Regarding the INF-γ, the results found that 30 (57.7%) who were positive for INF-γ were positive to IgG, while 1(33.3%) who were positive for IgG were negative for INF-γ. The difference was insignificant (P= 0.408).

Table (9): Association of anti-brucella IgG with cytokine variables.

cytokine variables		Anti-brucella IgG				P value
		Positive		Negative		
		No	%	No	%	
IL-17	Positive	21	65.6	11	34.4	0.102
	Negative	10	43.5	13	56.5	
IL-18	Positive	12	44.4	15	55.6	0.080
	Negative	19	67.9	9	32.1	
INF-γ	Positive	30	57.7	22	42.3	0.408
	Negative	1	33.3	2	66.7	

Significant difference between percentages using Pearson Chi-square test (χ^2-test) at 0.05 level.

Discussion:

Undoubtedly the importance of the current study is attributed to many aspects; the most importantly, the high prevalence of brucellosis as an endemic zoonotic disease in both Iraqi population and domesticated reproductive animals (Shareef, 2006). In this regard it has been reported that human brucellosis is endemic in most of the ME countries with Syria, Iraq, Saudi Arabia, Turkey, and Iran having the world's highest incidence rates (Pappas et al., 2006; Dean, et al., 2012). The annual incidence rate of human brucellosis in Iraq was 2.6/100000 population (Bagheri et al., 2020). On the animal side, It has been documented that the overall seroprevalence of brucellosis in food-producing animals over a period of 40

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

years was 14.14%, including 14.46% for sheep, 12.99% for goats, 11.69% for cattle, and 22.64% for buffalo with an increment rate of 9 times between 1979 and 2019 (Dahl, 2020).

It is worthy to mention the critical importance of small ruminants in the food supply with their considerable contribution to the provision of dairy products, meat, wool and hair for Iraqi's (Maxwell and Bill, 2008). For this reason, the people were routinely breed such food-producing ruminants in or near their houses to make benefit of their products. Furthermore, farmers had a custom to slaughter animals by themselves in their houses whenever they need meat or traditionally during religious or social occasions. Actually, this explain the higher positivity rate of brucellosis among people who practice home breeding of such animals in rural and semi urban areas (P= 0.099). These customs which were away from veterinary supervision are responsible for the significantly higher positivity rate of brucellosis among those traditionally use raw milk and meat obtained in the current study (P= 0.0001). In addition to consumption of raw milk and meat, in such home breeding, the farmer or probably members their family are aiding animals during delivery or abortion without safety precautions. Such high risk practices may responsible for the significantly higher positivity rate of brucellosis among family members or the clustering of brucellosis among Iraqi families (P= 0.045). Indeed, this fascinating result obtained in this study is largely attributed to the complete or partial absence of veterinary services, neglection of brucellosis as an important endemic zoonotic disease, beside the low health education and low living standard that promote perpetuation of the disease in Iraqi community (Shareef, 2006; Rossetti et al., 2017).

The successful intracellular stealthy lifestyle of brucellae has evolved through multiple strategies that maintain its reproduction and survive in the host cells for a long time, which eventually leads to the formation of chronic persistent infection (Ahmed et al., 2016; Jiao et al., 2021). More precisely, brucellae behave as a latent infection rather than chronic infection which primarily infects people at different ages, complete its traditional clinical course and then latently reside in different host cells with the propensity to reactivate annually causing most probably different clinical syndrome. These facts explain that 20% of patients included in the current study had repeated attack of brucellosis over the time. The traditional clinical picture of brucellosis including fever, night sweat and joint pain, are all highly associated (P= 0.0001) in patients who at least reported a new or primary infection. These results are consistent with most clinical studies conducted locally or elsewhere (Shareef, 2006; Dean et al., 2012; Matthew et al., 2013; Bagheri et al., 2020).

Rose Bengal rapid test was used as an inclusion criteria for enrollment of patients in this study. It is well known that Rose Bengal test is so fast, but it has many false-negative results during the chronic brucellosis (Roushan et al., 2005). Serum agglutination test is the most common acceptable serological diagnostic test for human brucellosis. Undoubtedly, in endemic areas the use of serum agglutination test titer $\geq$ 1:320 is more appropriate. It is necessary to explain that definitive treatment of patients has a correlation in declining serum agglutination test titers (Roushan et al., 2010). Keeping in this line, the significantly higher association of anti-brucella IgM (P= 0.003) and anti-brucella IgG (P= 0.0001) among patients compared to controls are not unusual since the patients were enrolled in this study based on their Rose-Bengal test positivity. These results are consistent with other local or abroad (Ruiz-Mesa et al., 2005; Hasanjani and Ebrahimpour, 2015). Indeed, IgM antibodies were the first to appear following infection and rise gradually during the course of acute infection. In contrast, IgG antibodies appeared later after the onset of infection (Elfaki et al., 2015). IgG antibodies may persist for many months after the successful antibiotic therapy. This explains the high seroprevalence of anti-brucella antibodies found in areas of endemicity and among individuals who had repeated flare up of brucellosis (Ariza et al., 1992). Moreover, 11 (20%) of patients were appeared to have positive IgM and IgG simultaneously. Such overlapping of IgG and IgM is atypical characteristic of latent infection, since the IgG antibody was remain in the host body due to the existence of the pathogen in the body as if it is in dormant state. During reactivation of such latent infection, the IgM is transiently elevated and decline rapidly followed by four fold increase in the IgG titers which persist for long period (Thakur et al., 2019).

Regarding the positivity rate of interleukins, only INF-γ was found to be significantly higher among brucellosis patient, while the IL-17 and IL-18 were slightly changed. However, basically, cytokine levels in acute or chronic brucellosis is a marker of immune response and an indicative marker of severity of brucellosis (Lin et al., 2020).

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

It is worthy to mention that most of the previous studies affirm the elevation of INF-γ with its effective clearance of brucellae through the secretion of IFN-γ, IL-2, and TNF-α by T helper 1 cells (Dornand et al., 2002; Pasquali et al., 2002; Celli, 2006). Since IFN-γ-producing Th1 CD4+ T cells for the control of brucella infection, whereas IFN-γ-producing CD8+ T cells or B cell-mediated humoral immunity which plays a role in the clearance of bacteria during primary infection (Wenpeng et al., 2013). Thus the current result is concordant with other studies which reported a higher levels of IFN-γ during brucellosis (Akbulut et al.,2007; Rodriguez-Zapata et al.,2010). Furthermore, It has been suggest that endogenous IL-10 promote effector functions of macrophages to control intracellular brucellae with significant increase of IFN-γ and IL-4 and that the production of the Th1 cytokine IFN-γ during brucella infection is also associated with a specific IgG3 and IgG2a response against the lipopolysaccharide antigen (Fernandez-Lago et al., 1996).

The IL-17 cytokine played a principal roles in both innate and adaptive immunity. So through its existence in the lamina propria and oral cavity mucosa, it play an essential role against infection through gastrointestinal tract (Marks and Craft, 2009). Therefore, IL-17 is a critical determinants of risk, severity or protection against brucellosis although it level decreased in patients at the end of treatment (Park et al., 2005; Sofian et al., 2016). The present result is consistent with that of (Ghaznavi-Rad et al., 2017) who reported insignificant increase in IL-17 suggesting that the cytokine production varies in the different stages of brucellosis.

It has been found that combined inoculation of IL-12 and IL-18 reduced the number of B. abortus in the spleen and that the effect of the treatment was mediated by an increased capability of spleen cells to produce INF- γ at the early phase of infection (Pasquali et al., 2002). Conversely, once the infection was established, B. abortus selectively limits IL-18 secretion without affecting endogenous IFN- γ production (Fernandez-Lago et al., 2005). Similar elevated levels of IL-18 was reported in acute brucellosis (Kayhan et al., 2016).

References:

1. Ahmed, W.; Zheng, K. and Liu Z. Establishment of Chronic Infection: Brucella's Stealth Strategy. Front. Cell. Infect. Microbiol. 2016; 6:30.
2. Akbulut, H.; Celik, I. and Akbulut, A. Cytokine levels in patients with brucellosis and their relations with the treatment. Indian J.Med. Microbiol. 2007; 25(4), 387.
3. Ariza, J.; Pellicer, T.; Pallares, R.; Foz, A. and Gudiol, F. Specific antibody profile in human brucellosis. Clin. Infect. Dis. 1992; 14:131–140.
4. Bagheri, N.R.; Krecek, R.C.; Khalaf, O.H.; Hailat, N. and Arenas-Gamboa, A.M. Brucellosis in the Middle East: Current situation and a pathway forward. PLoS Negl. Trop. Dis.2020; 14(5): e0008071.
5. Bedoya, S.K.; Lam, B.; Lau, K. anf Larkin, J. Th17 cells in immunity and autoimmunity. Clin. Dev. Immunol. 2013; 2013:2013-16.
6. Celli, J. (2006). Surviving inside a macrophage: the many ways of Brucella. Research in microbiology, 157(2), 93-98.
7. Corsetti, P.P.; de Almeida, L.A.; Carvalho, N.B.; Azevedo, V.; Silva, T.M.; Teixeira, H.C.; et al. Lack of Endogenous IL-10 Enhances Production of Proinflammatory Cytokines and leads to Brucella abortus clearance in mice. PLoS ONE, 2013; 8(9): e74729.
8. Dahl, M.O. Brucellosis in food-producing animals in Mosul, Iraq: A systematic review and meta-analysis. PLoS ONE 2020; 15(7): e0235862.
9. de Figueiredo, P.; Ficht, T.A.; Rice-Ficht, A.; Rossetti, C.A. and Adams, L. G. Pathogenesis and Immunobiology of Brucellosis: Review of Brucellae Host Interactions. Am. J. Pathol. 2015; 185: 1505e1517.
10. Dean, A.S.; Crump, L.; Greter, H.; Schelling, E. and Zinsstag, J. Global Burden of Human Brucellosis: A Systematic Review of Disease Frequency. PLoS Negl. Trop. Dis. 2012; 6(10): e1865.
11. Dornand, J.; Gross, A.;Lafont, V.; Liautard, J.; Oliaro, J. and Liautard, J. The innate immune response against Brucella in humans. Vet. Microbiol. 2002; 90(1–4): 383-392.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

12. Elfaki, M.G.; Alaidan, A. A.; Al-Hokail, A. A. Host response to Brucella infection: review and future perspective. J. Infect. Dev. Ctries. 2015; 9(7):697-701.
13. Elfaki, M.G.; Al-Hokail, A.A.; Nakeeb, S.M. and Al-Rabiah, F.A. Evaluation of culture, tube agglutination, and PCR methods for the diagnosis of brucellosis in humans. Med. Sci. Monit. 11: 2005; MT69-74.
14. Fernandez-Lago, L.; Orduna, A.and Vizcaíno, N. Reduced interleukin-18 secretion in Brucella abortus 2308-infected murine peritoneal macrophages and in spleen cells obtained from B. abortus 2308-infected mice. J. Med. Microbiol. 2005; 54(Pt 6):527-31.
15. Fernandez-Lago,L., Monte, M. and Chordi, A. Endogenous gamma interferon and interleukin-10 in Brucella abortus 2308 infection in mice. FEMS Immunol Med Microbiol. . 1996; 15(2-3):109-14.
16. Gaffen, S.L. An overview of IL-17 function and signaling. Cytokine. 2008; 43e:402-407.
17. Ghaznavi-Rad, E.; Khosravi, K.; Zarinfar, N. and Mosayebi, G. Reduced IFN-γ production in chronic brucellosis patients. Iranian J. Immunol. 2017; 14(3), 215-222.
18. Hasanjani, R.M.R. and Ebrahimpour, S. Human brucellosis: An overview. Caspian J. Intern. Med. 2015; 6(1): 46-47
19. Isailovic, N.; Daigo, K.; Mantovani, A. and Selmi, C. Interleukin-17 and innate immunity in infections and chronic inflammation. J. Autoimmun. 2015;60:1-11.
20. Jiao, H.; Zhou, Z.; Li, B.; Xiao, Y.; Li, M.; Zeng, H.; Guo, X.and Gu, G. The Mechanism of Facultative Intracellular Parasitism of Brucella. Int. J. Mol. Sci. 2021, 22: 3673.
21. Kayhan, B.; Kayabas, U.; Kolgelier, S.; Otlu, B.; Gul, M.; Kurtoglu, E. L. and Bayindir, Y. (2016). Mystery of Immune Response in Relapsed Brucellosis: Immunophenotyping and Multiple Cytokine Analysis Running . Mediterranean Journal of Infection Microbes and Antimicrobials.
22. Lin, Z. Q.; Lin, G. Y.; He, W. W.; Zhang, C.; Zhang, R.; Li, Y. D.; et al. (2020). IL-6 and INF-γ levels in patients with brucellosis in severe epidemic region, Xinjiang, China. Infectious diseases of poverty, 9, 1-6.
23. Mantur, B.G.; Biradar, M.S.; Bidri, R.C.; Mulimani, M.S.; Veerappa, K.; Kariholu, P.; et al. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. J Med Microbiol 2006; 55: 897-903.
24. Marks, B.R. and Craft, J. Barrier immunity and IL-17. Semin Immunol. 2009; 21:164–71.
25. Matthew, P.; Rubacha,, E.B.; Halliday, S.C. and Crumpa, J.A. Brucellosis in low-income and middle-income countries. Curr. Opin. Infect. Dis. 2013; 26(5): 404–12.
26. Maxwell, J.R. and Bill, D,E. Developing a brucellosis public health information and awareness campaign in Iraq. Military medicine. 2008; 173(1):79–84.
27. Pappas, G.; Papadimitriou, P.; Akritidis, N.; Christou, L. and Tsianos, E.V. The new global map of human brucellosis. Lancet Infect. Dis. 2006; 6(2):91–99.
28. Park, H.; Li, Z.; Yang, X.O.; et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nature Immunol. 2005; 6:1133-1141.
29. Pasquali, P.; Adone, R.; Gasbarre, L.C.; Pistoia, C. and Ciuchini, F. Effect of Exogenous Interleukin-18 (IL-18) and IL-12 in the Course of Brucella abortus 2308 Infection in Mice. Clinc. Diag. Lab. Immunol. 2002; 9(2): p. 491–492
30. Pasquevich, K.A.; Ibanez, A.E.; Coria, L.M.; Garcia-Samartino, C.; Estein, S.; Zwerdling, M.; et al. An oral vaccine based on U-Omp19 induces protection against B. abortus mucosal challenge by inducing an adaptive IL-17 immune response in mice. PLoS ONE. 2011; 6:e16203.
31. Rodríguez-Zapata, M.; Matías, M. J.; Prieto, A.; Jonde, M. A.; Monserrat, J.; Sánchez, L.; et al. Human brucellosis is characterized by an intense Th1 profile associated with a defective monocyte function. Infect.Immun. 2010; 78(7), 3272-3279.
32. Romagnani, S. Human Th17 cells. Arthritis Res. Ther. 2008; 10:206.
33. Rossetti, C.A.; Arenas-Gamboa, A.M. and Maurizio E (2017) Caprine brucellosis: A historically neglected disease with significant impact on public health. PLoS Negl. Trop. Dis.2017; 11(8): e0005692.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

34. Roushan, M.;R, Amin, M.J.; Abdoel, T.H. and Smits, H.L. Application of a user-friendly Brucella-specific IgM and IgG antibody assay for the rapid confirmation of Rose Bengal-positive patients in a hospital in Iran. Trans. Roy. Soc. Trop. Med. Hyg. 2005; 99: 744-50.
35. Roushan, M.R.; Amiri, M.J.; Laly, A.; Mostafazadeh, A. and Bijani, A. Follow- up standard agglutination and 2- mercaptoethanol tests in 175 clinically cured cases of human brucellosis. Int. J. Infect. Dis. 2010; 14: 250-3.
36. Ruiz-Mesa, J.D.; Sánchez-Gonzalez, J.; Reguera, J.M.; Martín, L.; Lopez, P.S.; Colmenero, J.D. Rose Bengal test: diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clin Microbiol Infect 2005 11:221–5.
37. Shareef, J.M. A Review of Serological Investigations of Brucellosis among farm Animals and Humans in Northern Provinces of Iraq (1974–2004). J. Vet. Med. 2006; 53(s1):38–40. .
38. Sofian, M.; Ramezani, A.; Mousavi, A.; Banifazl, M.; Cherei, S.; Cherei, A. and Aghakhani, A. Interlukine-17 and TGF-ß levels in patients with acute brucellosisbefore and after treatment. Turkish journal of medical sciences, 2016; 46(5), 1348-1352.
39. Thakur, A.; Mikkelsen, H.; and Jungersen, G. Intracellular Pathogens: Host Immunity and Microbial Persistence Strategies. J. Immunol. Res. 2019, ID 1356540: 24 pages.
40. Vitry, M.A.; De Trez, C.; Goriely, S.; Dumoutier, L.; Akira, S.; Ryffel, B.; et. al. Crucial Role of Gamma Interferon-Producing CD4 Th1 Cells but Dispensable Function of CD8 T cell, B cell, Th2, and Th17 Responses in the Control of Brucella melitensis Infection in Mice. Infection and Immunity 2012; 80 (12): 4271– 80.
41. Wenpeng, G.u.; Wang, X.; Qiu, H.; Cui, B.; Zhao, S.; Zheng, H.; et al. Comparison of Cytokine Immune Responses to Brucella abortus and Yersinia enterocolitica Serotype O:9 Infections in BALB/c Mice. Infect. Immun. 2013; 81 (12): 4392– 8.
42. Xavier, M.N.; Winter, M.G.; Spees, A.M.; Nguyen, K.; Atluri, V.L.; et al. CD4+ T Cell-derived IL-10 Promotes Brucella abortus Persistence via Modulation of Macrophage Function. PLoS Pathog, 2013; 9(6): e1003454.
43. Yasuda, K.; Nakanishi, K. and Tsutsui, H. Interleukin-18 in Health and Disease. Inte. J. Mol. Sci.2019; 20 (3): 649.