Detection Malta Fever by Interferon-gamma and Steroid Hormone S Level

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Abstract

Malta fever is one of the most common bacterial zoonosis, its causes abortion of pregnant women. Abortion is the chief obvious manifestation of *Brucella* infection. *Brucella* like better cattle placenta as a result of great concentration of erythritol sugar, whereas human placenta there is no erythritol sugar only steroid hormone, for this reason designed the our project . In this study, 100 aborted women were included , where referred to maternity and children hospital of Babylon province \ Iraq. Diagnosis of Brucella infection in these abortions was concentrated on serological and bacteriological technique. Serological studies included the use of RB and ELISA tests. Aggressive differences between RB and ELISA results have been shown. Brucella isolated and identified from aborted placenta and blood samples were 7 (7%) isolates from aborted women.

Hormonal assessment by Immunohistochemical technique in *Brucella* infected women, showed significant decrease in progesterone expression in comparison with that aborted due to other causes, in other hand *Brucella* infected women showed high expression in estrogen hormones . ELISA technique was the valuable serological test to confirm the diagnosis of brucellosis as compared with RB test.

Keyword: *Malta fever , Brucella , Interferon-gamma , Steroid hormone*

Introduction

Malta fever also called Brucellosis, is a zoonotic disease effecting humans and animals in many countries e.g, Mediterranean area, Iraq, middle east, India, and America[(1)] . Reports from the regions where *Brucella melitensis* infection is endemic, propose that there is an increased rate of abortion in asymptomatic pregnant women. The diagnostic method acknowledged to produce the best results in terms of specificity is the isolation of *Brucella* organisms from the suspected human , Different *Brucella* species are recognized as causative agents of brucellosis and some of them are acknowledged to be pathogenic to humans [(2-8)] . However, Because of the variety of the disease and its non-specific clinical manifestation, the clinical diagnosis of Malta fever remains a challenge. Malta fever (Brucellosis) mimics other infectious and non-infectious diseases, resulting in a delay in diagnosis or misidentification of the disease[(9)]. The diagnosis is importantly dependent on a epidemiological history , clinical signs , patient's medical, biochemical testing , hematological, radiological examination and, necessarily, on *Brucella*-specific laboratory tests. Very important sides for cporrect and fast diagnosis are the disease-specific laboratory tests and knowledge of their weaknesses, proper analysis and correct assessment of their results [(10)]. Rose Bengal (RB) is the main serological test used to identify antibodies against brucellosis. Because of the difficulties in the method and low sensitivity of the isolation methods, laboratory diagnosis relies largely on serological tests. The major antigens of *Brucella* being used in serological testing are the internal-cytosolic proteins and lipopolysaccharide (smooth-S LPS) [(11)]. *Brucella* Lipopolysaccharide is a strong immunogen but its epitopes are the chief etiology of cross-reactions with other Gram-negative bacteria , a condition that creates evaluation more difficult[(12)]. Cytokines have an important role in the pathogenesis of Malta fever and the Th1/Th2 balance may include in the resistance or susceptibility to the disease [(13)]. As the pathogen is intracellular, Th1 and its linked cytokines

are responsible for control of the infection. Experimental studies showed that IFN-γ is essential for exclusion of *Brucella* and for host survival in case of virulent *Brucella* challenge [14].The aims of the present work were:

1- tropism of *Brucella* to uterus of women that it not has erythritol sugar

2- Evaluate steroid hormones level in placenta and blood is specific method to detection of Malta fever .

3- Evaluate interferon-gamma level in blood.

Materials and Methods:

Human brucellosis kit was provided by Elabscience /China. Gram's stain solution. Rose Bengal antigen was provided by Omega company, UK. antibrucella abortus , antibrucella melitensis and Monospeciic antiserum were supplied by Difco, USA.

Methods

Samples collection.

1-Clinical signs of aborted women were recorded by physician to show pregnancy period and sings of abortion.

2-Blood samples: Hundred blood samples from women were obtained. serum samples have been obtained for serological and immunological assessments using RB and ELISA tests.

3-Aborted Placentas:

Hundred placenta samples from aborted women have been obtained, at maternity and children hospital of Babylon province, placenta samples were cultured directly on *Brucella* selective medium and blood agar.

4-Immunohistochemistery for placenta specimens was performed as described by (Dako, UK), The expression of "estrogen and progesterone hormones were measured as the same scoring system" used by Mao *et al*., (15). The positivity of cells for expression of hormones were seen as brown staining. It was graded as four grad of the cells staining positive for hormones.

Score	0	1+	2+	3+	4+
Positive Cells	<10%	10-25%	25-50%	50-75%	>75%

5- IFN-gamma ELISA performed as described by (RayBiotech. Inc).

Statistical Analysis:

The results are expressed at percentage by Chi-square test, (SPSS) for Windows program was used to compare between the frequencies. Student t test was used to compare between means of groups. The significance was accepted as P value < 0.05 .

Results

Clinical signs in women included fever and bleeding, most women infected with *Brucella* were aborted at first stage of pregnancy .

Serological tests:

ELISA , RB test revealed that RB results were positive in 27 cases (27%). ELISA results were positive in 14 cases(14%) . according to Pearson Chi-Square test, the difference in RB and ELISA positive cases was significant (P<0.05) show in Table (1) .

Table(1) showed the diagnostic test of *Brucella* infected women

Blood samples	RB	%	Culture(+)	%	ELISA	%
100	27	27	7	7	14	14

Bacterial isolation & identification:

Out of the 100 aborted women, 7 (7%) were positive for culture, from 14 patient blood samples that positive for positive ELISA, 7 isolates were positive by culture, after 2-4 days the *Brucella* culture recognized on the basis of colonial morphology (translucent , round with pearly appearance).

Isolates from blood and placenta samples were Gram-negative, coccobacilli, arranged singly in short chain or small groups stained with modified ziehl-

neelsen stain , Biochemical test of *Brucella* show in table (2)

Table (2): Biochemical test of *Brucella*

	Test	Results
1	Haemolysis	-
2	macconkey agar	-
3	indol	-
4	MR-VP	-
5	gelatinase	-
6	Citrate utilization	-
7	urease	+
8	nitrate reduction	+
9	catalese	+

Steroid hormones assessment by immunohistochemistry assay:

Result of imunnohistochemical assay showed positive staining for progesterone hormone in placenta of aborted woman non infected with *Brucella* , compared with that of positive for *Brucella* infection which exposed low intensity for IHC staining of progesterone, the score differences were also seen in(table 3).

Table (3) : Existence of progesterone molecule in placenta of aborted women .(IHC assay)

Score	Positive for Brucella infection		Negative for Brucella Infection	
	NO	%	NO	%
1	3	42.58	0	0
2				
3	4	57.14	1	14.28
Tatal of negative score	*100%		14.28%	
4	0	0	1	14.28
5	0	0	5	71.42
Tatal of positive score	0%		85.71%	

*Significant ($p \leq 0.05$)

According to study the estrogen particles . there is obvious rise for estrogen stain for placenta tissue through *Brucella* infection . as determined by staining of biopsies , the immune staining of estrogen were positive at high level in 85.71% (6 out of 7) in *Brucella* infected patients, with highly statistical association ($p \leq 0.05$) between the infected & non infected groups (table 4).

Table (4) : Amount of estrogen molecules in placenta of aborted women (IHC assay)

Score	Negative for Brucella infection		Positive for Brucella Infection	
	NO	%	NO	%
1	0	0	5	71.42
2	2	28.57	1	14.28
3	5	71.42	1	14.28
Score ;1< 25% ; 2(25-74)% ; (75-100)%				

Interferon-gamma assessment by ELISA technique

Patients with Malta fever had significantly (P<0.05) higher serum levels of IFN-γ (175.078 ± 69.821pg/ml) compared to control group (39.358 ±29.847 pg/ml) show in (Figure 1).

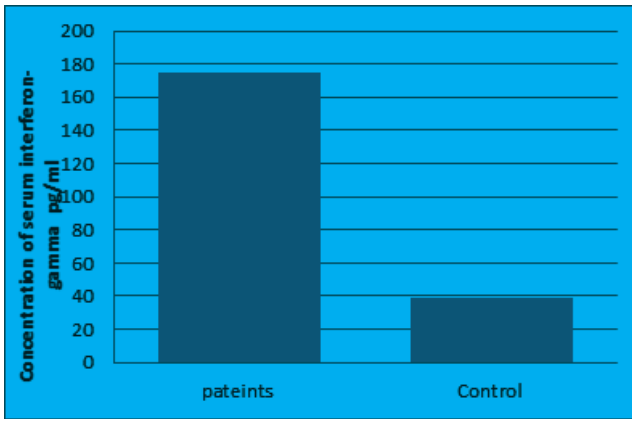

Figure 1 : Serum concentration of IFN-γ in patients and control

Discussion

RB gave positive result in aborted women but were negative for ELISA and bacterial isolation infection, because the RB test is rapid and screening test and may be give cross-reaction with other Gram negative bacteria example : *E.coli* O:157, *Yersinia enterocolitica* O:9, *Vibrio cholerae* O:1, *Salmonella spp., Francisella tularensis and Pseudomonas maltophilia,* and give false positive for RB [16].

Antibodies to *Brucella* appear in the serum within (1-2) weeks of infection. The initial response is the appearance of IgM isotype (which can be easily detected by RB) followed by a switch to IgG, after a while titers of both Immunoglobulins classes increase distinct most of the usual serological tests, ELISA is effective in distinguishing all immunoglobuline (antibodies) classes and sub-classes essential in diagnosis and appears to be the most sensitive serological test increase in IgG but not IgM. IgA titres roughly paralleled IgG titers[17]. ELISA using S-LPS Ag can be used to measure the development of immunoglobulin isotypes following infection and after treatment[18]. Steroid hormones concentration in aborted women positive for brucellosis were showed decrease in progesterone levels and increase in other hormones due to *Brucella* infection, aborted women negative for brucellosis notice significant increase in hormone , this certified that other cause of abortion may be not effect on progesterone synthesis.

Estimation of hormones are more effective method to conformation of *Brucella* infection, while other serological methods may be causes cross-reaction and false positive results.

There are two HSD3B1 proteins, labeled type I and type2 that are expressed by different genes and function in different regions of the body[19]. HSD3B1has too been shown to be there a highly specific and sensitive trophoblast-associated marker, also showed that expression of 3β-HSD in trophoblast more than 50% considered as positive cells for 3βHSD [20]. To form steroid hormones, the subsequent processing of pregnenolone requires enzymes related to smooth endoplasmic reticulum, such as 17 α-hydroxylase (P450c17α) and 3β-hydroxysteroid dehydrogenase/ isomerase (3βHSD) pregnenolone is converted to progesterone by the enzyme 3β-HSD, which in turn, is converted to androstenedione by the enzyme 17α-hydroxylase/C17, 20 – lyase (P450c17α) [21].

In this study, have high serum levels among patients with Malta fever compared with control. These results are in agreement with earlier studies [22] who reported height this cytokine in Malta fever patients.

Most studies specified that CD4+ T lymphocytes are the main 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 [23]. As there are relatively high serum levels of IFN-γ, it indicates an enlarged number of CD4+ which, in turn, indicates a chronic infection" [24].

Conclusion

. In conclusion, placental immunohistochemical and assessments for steroid hormones (progesterone) and interferon-gamma have an efficient diagnostic values which can be included for confirmation of brucellosis .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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