Imunocytochemistry Detection of M1CA Gene Receptor in Human Patients with Brucellosis

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Abstract:
Background: The most relevant cytokine involved in the response against the genus Brucella is interferon gamma (IFN-γ) that is mainly produced by NK cells and stimulated by the binding between their receptors NKG2D receptor and its ligand M1CA gene expressed on Brucella – host infected cells.

Aim: To evaluate the relationship between host susceptibility for Brucella infection and the expression of MICA gene which demonstrated by imunocytochemistry staining technique.

Methods: Rose Bengal test was applied for 340 patient serum then Brucella blood culture was carried out for Rose Bengal positive samples. Imunocytochemistry technique was used to check the expression of MICA gene receptor on human infected cells. Moreover, the level of IFN-γ was measured in patient sera by using of ELISA technique.

Results: Rose Bengal Test (RBT) revealed that 155 patients were positive. Brucella- Blood culture revealed that only eight samples gives positive for blood culture. Five samples expressed MICA gene receptors. The level of IFN-γ revealed a significant increasing (p ≤ 0.05 ) in positive samples for MICA gene receptors as compared to negative samples for MICA gene receptors.

Conclusion: There is a good relationship between the expression of MICA gene receptor and immune response against Brucella that represented by an increasing of IFN-γ.

Key words: Human Brucellosis, MICA gene receptor, IFN-γ.

Introduction:
Brucellosis is a zoonotic disease affecting animals and man in many parts of the world, animals are considered as natural reservoir of human Brucellosis, (Young, 1995 ; AKina et al., 2010). Brucellosis is a multi-system disease that is endemic in many Mediterranean countries. The disease is transmitted to human by consumptions of infected unpasteurized dairy products, aerosol or by direct contact with infected animals (AKinci et al., 2001), or through ingestion of uncooked meat Meantime (Young, 1995). Occupational disease is contracted by exposure of abattoir workers and veterinarians to infected animals especially aborted fetuses, fluid, membrane, or urine (Ropson et al., 1993).

Immune response to Brucella infection depends mainly on cell mediated immunity and to a lesser extent humeral immunity. Cell mediated immunity play an a critical role in Brucella infection (Montaraz et al., 1986; Raya et al., 1989). Macrophages, neutrophils, natural killer cells, and γδT cell are dependable for the innate immunity in the early phase of infection, and adaptive immunity against Brucella depends on α β T cells (Araya et al.,
1989). Brucella organism induces production of antibody, this can give some protection against secondary infection, but is not believed to be a chief contribution to healing from primary infection (Baviov et al., 1982).

According to (Heremans et al., 1963; Corbeil et al., 1988), explained low concentrations of IgM or IgG antibodies in naturally infected species appear to promote lysis of Brucella by the classical complement pathway. While Higher of IgG antibody levels present during active infection likely promotes bacterial intracellular localization and phagocytosis by macrophages (Hoffmann and Houle, 1995). Intracellular numerous cell surface component of Brucella has been assessed significant activity to stimulate immune response (Oliveira and Splitter, 1996). Interferon-gamma (IFN-γ) plays an important role in mediating resistance to primary and secondary Brucella infection (Baldwin, 1993; Zhan and Cheers, 1993). T lymphocytes from untreated brucellosis patients are activated in vivo and show Th1 cytokine production polarization, with strikingly high serum IFN-γ levels (Rodríguez-Zapata et al., 2010). As with immunity to other intracellular pathogens, immunity to B. abortus depends on antigen-specific T-cell-mediated activation of macrophages, which are the major effectors facilitating killing and inhibiting replication of Brucella. The Th1 cell induced - cytokines, like IFN-γ, are important for the ctivation of macrophages and in resistance to in vivo and in vitro Brucella infections (Zhan et al., 1993).

gamma interferon (IFN-γ) induces macrophage activation and intracellular killing of Brucella infection in animals model (Copin et al., 2007). MICA gene receptor is a member of the « major histocompatibility complex (MHC) class I chain-related genes » or MIC family, MICA function MICA gene encodes a cell surface highly glycosylated protein that is expressed exclusively in the basolateral membrane of intestinal epithelium cells, and epithelium-derived tumours (Groh, 1996; Groh, 1999). MICA gene with Brucella was played important role at susceptibility and protection in human Brucellosis. The MICA chain is stress-induced when infection with Brucella occurs in acute or chronic cases. The mechanisms by which Brucella evades intracellular killing are incompletely understood. Nevertheless, Brucella organisms ultimately become sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as lymph nodes, liver, spleen and bone marrow. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate, the disease is termed “localized”. Commonly, localization involves organs of the RES. Macrophages are the first target of Brucella invasion, and the bacteria can survive within this naturally hostile intracellular environment (Pizarro-Cerda, 1998). Macrophages are significant in transporting Brucella to tissue throughout the host, where they can survive in a variety of cell types (Arenas, 2000). Poor diagnosis and lack of treatment can result in life-threatening complications (Boschirolie et al., 2001).

This study aimed to illustrate the role of MICA gene in the susceptibility of host to Brucella infection based on the level of IFN-γ which is considered as the marker for cell-mediated immunity against Brucella.

Materials and methods

Subjects:

Human patients: A total of (340) blood samples clinical blood samples were collected from patients suffering from Brucellosis that diagnosed through clinical signs and symptoms. Patients were admitted to seven hospitals in IRAQ: Babylon Hospital for Maternal and Pediatrics, Al-Hilla Surgical Teaching Hospital, Al-Qassim general Hospital, Al-Hashimia
general Hospital, Marjan Hospital, Central health laboratory, and private labs during the period from Dec. 2010 to Jan. 2012.

**Controls:** Thirty persons (17 male & 13 females) apparently healthy individuals were negative for PRT & wed as control group.

**Rose Bengal test:** Rose Bengal test (RBT) was carried out for all the samples as recommended by (OIE, 2009).

**Brucella culture:** Brucella culture was applied for patients with positive results for RBT by using of freshly drawn blood samples (5 ml) according to (Alton., 1998). Molecular Brucella identification by using the primers(B4/B5) specific for *B. melitensis* and *B. abortus* based on (Baily et al., 1992).

**Detection of MICA gene receptor:** Lymphocyte separation and slide preparation according to Isopaque-ficol technique originally described by (Boyum, 1968; Marian and Catherine, 1998).

**Kits used for immunocytochemistry staining techniques.**
1-Mouse specific HRP/DAB detection IHC Kit. (ab64259) company / abcam/UK, as following components. (table1).
2-Mouse monoclonal [1D11] to NKG2D kit.(ab35033) company / abcam /UK

Table (1) components detection IHC Kit

<table>
<thead>
<tr>
<th>components</th>
<th>Identifier 15ml</th>
</tr>
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<tbody>
<tr>
<td>ab64259</td>
<td>1×1 unit</td>
</tr>
<tr>
<td>Hydrogen Peroxide Block</td>
<td>1×15ml</td>
</tr>
<tr>
<td>Protein Block</td>
<td>1×15ml</td>
</tr>
<tr>
<td>Biotinylated goat anti-mouse IgG (H+L)</td>
<td>1×15ml</td>
</tr>
<tr>
<td>Streptavidin Peroxidase</td>
<td>1×15ml</td>
</tr>
<tr>
<td>50×DAB Chromogen</td>
<td>1×0.5ml</td>
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</tbody>
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**Gamma interferon estimation:** The cellular immune response for patients was evaluated by estimation of IFN-γ using ELISA method as recommended by manufacture company (Ray Biotech, USA).

**Results and Discussion:**

**Brucella isolation and identification:** 155 patients out of 340 patients were positive for RPT, eight of which were positive for blood culture.

When, blood culture carried out for patients positive for RBT, only 8 of them gives Six of positive blood culture were diagnoses as *B. melitensis* where as two of them were dignosed as *B. abortus* by PCR technique. Several studies indicated the role of PCR as a golden standard for the identification of *Brucella* (Al-Nakkas et al., 2005 and Al-Ouqaili, 2006).

**M1CA gene receptor Assay:** Demonstration of M1CA gene was carried out for 8 patients with positive culture for *Brucella* there is a significant differences *(p≤0.05)* between the patients expressed MICA gene receptor and patients without MICA gene receptor table(2).

Table(2) Scoring of binding between M1CA gene receptor and NKG2D ligand for positive patients with Brucellosis.

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Scoring positive Immunocytochemistry</th>
<th>Types of Brucella Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8%</td>
<td><em>B. melitensis</em></td>
</tr>
<tr>
<td>2</td>
<td>11.1%</td>
<td><em>B. melitensis</em></td>
</tr>
<tr>
<td>3</td>
<td>7.6%</td>
<td><em>B. melitensis</em></td>
</tr>
<tr>
<td>4</td>
<td>6.6%</td>
<td><em>B. melitensis</em></td>
</tr>
</tbody>
</table>
Five patients were positive for M1CA gene receptor assay according to the estimated scores of binding between M1CA gene receptor & KNG2D legend dor positive patients with brucellosis (table 2) indicated by the presence of brown color on the surface of lymphocytes (figure 1) or the whole of host T-lymphocytes and NK cells (figure 2). A control group showed, no staining and no binding score with immunoperoxidase staining. The stained cells were blue in color which indicated for negative result figure (2) white arrow.

Figure(1): lymphocytes, lymphocyte-specific stain(black arrow), the NKG2D receptors are stained with immunoperoxidase stain, white arrow is negative 1000 X.
Figure(2): lymphocytes, lymphocyte-specific staining. Immunoperoxidase whole brown cells (black arrows), white arrows is negative, black arrows is faintly stain, 1000X.

According to our knowledge, no similar studies were carried out to investigate the MICA gene receptors in patients with Brucellosis in Iraq and Middle east region. Bravo, et al. (2007) studied the polymorphism of MICA gene for Brucella infection in human and showed that the polymorphism in MICA-A4 allele and MICA-A5 allele increase the susceptibility to Brucella infections. Moreover, in this study, the patients with positive Brucella culture and positive MICA gene receptor expressed normal immune response against Brucella represented by significant level of IFN-γ compared with negative patient for MICA gene receptor (table 3). Fernandes-Prada et al. (2001) indicated that MICA gene receptor plays a central role in the killing of Brucella. In 1997, Mizuki et al. Concluded that MICA is a possible candidate gene for the Behçet’s disease. Also Hepatitis B, Hepatitis C may be associated with MICA polymorphisms related with MICA15 (Aurélie Frigoul and Marie-Paule Lefranc, 2005). Junko Shojima et al., (2009). found that MICA is one of the promising candidate molecules that might be involved in susceptibility to Pulmonary Mycobacterium avium Complex Infection disease. Bing Mei et al., (2009) found that the MICA locus might modify host susceptibility to Chlamydia trachomatis infection.

The association of a specific MICA allele with C. trachomatis IgG antibodies among women with infertility. Kerrie Tosh et al (2006) study has provided strong evidence that there is a role for the truncated MICA protein, encoded by the MICA_5A5.1 allele, in leprosy susceptibility in South Indian families. where they are recognized by the NKG2D receptor on gd T cells, CD81 ab T cells and natural killer cells, all of which contribute to defense against Mycobacteria. Holger Kirsten., (2009) present evidence for linkage and association of MICA-250 (rs1051794) with RA independent of known hladrb1 risk alleles, suggesting MICA as an RA susceptibility. These findings make MICA an interesting candidate gene for association studies in RA.

Level of Gamma Interferon: the mean levels of IFN-γ estimated by ELISA technique were (17.716, 7.449, 5.25 Pico mol / ml), in patients with expression of MICA gene receptor, patients without expression of MICA gene receptor, and healthy controls respectively. The results indicated the good association between the expression of MICA gene receptor and the production of IFN-γ by TH1 cells and NK cells. There is no available data to compare the results among the three studied groups. However, results were compatible to a large extent to (Ahmed et al., 1999) found that IFN-γ and IL-12 increased in patients with brucellosis as well as the TH1 response is also activated. The IFN-γ plays a central role in the eradication of intracellular pathogens including Brucella by reducing the number in mice by inducing antimicrobial activity in macrophages. (Jiang, and Baldwing,. 1993). The level of IFN-γ was significantly higher (P≤0.05) in brucellosis-positive cases than in negative cases and controls.
The findings of the current work strongly indicated the role of MICA gene receptor in the resistance against Brucella infection which expressed by significant increasing of IFN-γ in patients with MICA gene receptor expression as compared with patients without expression of this receptor.

**References:**


