Histopathological study in liver and spleen of mice infected with *Brucella melitensis*

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Abstract

Brucellosis is a chronic infectious disease caused by *Brucella spp*.,a Gram-negative facultative intracellular pathogen that affects humans and animals, leading to significant impact on public health and animal industry. The mouse is the animal model more commonly used to study chronic infection caused by *Brucella*. This model is most frequently used to investigate specific pathogenic factors of *Brucella spp*. This work was done to study the histopathological in liver and spleen in mice infected with *Brucella melitensis*. A total of 20 female mice (8 week of age) a 10 mice control and 10 injected with 10^5cfu (colony forming unit) of *Brucella melitensis* by injection intraperitonial per animal. Samples (liver and spleen) were collected at 6 weeks period of infection and kept in 10% formalin study which revealed congestion, granulomatous , fibrosis in liver and increased number of lymphohistiocytic cells and increased amount of red pulp in spleen. This study concluded that the histopathological in liver and spleen caused by *Brucella melitensis* in mice are similar to those observed in humans with brucellosis, which indicated that the mice are suitable model for histopathological studies of human being diseases especially brucellosis.

Key word: *Brucella,* liver, spleen.

دراسة التغيرات النسيجية المرضية في كبد وطحال الفئران المصابة ببكتريا *Brucella militensis*

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الخلاصة

مرض الحمى المالطية Brucellosis من الأمراض المزمنة والمشتركة بين الإنسان والحيوان التي تسببه بكتريا سالبة لصبغة كرام وهي *Brucella sp.*. تعتبر الفئران من اكثر الحيوانات المختبرية استخداما في هذا الحقل للبحث عن المسببات المرضية لهذه البكتريا. تناول البحث دراسة تأثير الإصابة ببكتريا البروسيلا من جنس*Brucella militensis* ودرجة ضراوتها على أنسجة كلا من الكبد والطحال في الفئران. تضمنت الدراسة استخدام 20 من الفئران الإناث بعمر ثمانية أسابيع, 10 منها استخدمت كمجموعة سيطرة و10 أخرى تمت معاملتها ببكتريا البروسيلا حيث تم حقن 10^5 من عزلة نقية للبروسيلا في البريتون. جمعت العينات بعد مرور ست أسابيع من الحقن بعدها شرحت الحيوانات وجمعت الأعضاء (الكبد والطحال) والاحتفاظ بها في 10٪ من الفورمالين لغرض الدراسة النسيجية. أظهرت النتائج وجود تغيرات واضحة في أنسجة كل من الكبد والطحال تمثلت بظهور احتقان وأورام حبيبية وتليف في الكبد مع زيادة عدد خلايا النسيج اللمفي وزيادة نسبة اللب الأحمر في الطحال مع تجمع للخلايا الالتهابية. وخلصت هذه الدراسة إلى أن التغيرات النسيجية في كبد وطحال الفئران المصابة ببكتريا *Brucella militensis* مماثلة لتلك التي لوحظت في البشر مع الحمى المالطية. وفي السنوات

الأخيرة الماضية قد استخدمت النماذج الحيوانية, وخاصة الفئران، على نطاق واسع للحصول على معلومات قيمه تتعلق بامراضية هذه البكتريا في الجسم الحي.

الكلمات المفتاحية: البروسلا، الكبد، الطحال.

Introduction

Brucellae are gram-negative coccobacilli that are considered facultative intracellular pathogens capable to survive and replicate in phagocytic and nonphagocytic cells, establishing a chronic infection in both humans and animals (1). Infection causes a chronic disease termed brucellosis, the most important worldwide zoonosis (2). The genus consists of several species, differing from each other on the basis of the specific host they invade. Bacteria that are pathogenic to a variety of livestock animals and humans. Many species of wild animals including mice are susceptible to brucellosis and may serve as natural reservoirs of brucellosis for domestic animals and human beings (3, 4). The potential role of wild rodents as *Brucella* reservoirs was also reported by many authors (5, 6). Relevant aspects of *Brucella* pathogenesis have been intensively investigated in both cellular and animal models. The mice are the animal model most extensively used to study chronic infection caused by *Brucella spp* (7). The *Brucella* organism's predilection for organs rich in reticuloendothelial cells (spleen, liver, bone marrow, lymph nodes) and its intracellular location are responsible for the chronicity of the disease, which can last for months or even years (8). The lymphatic system is important in mounting an immune response to foreign antigens in humans and animal models. The liver and spleen were produced a large amount of lymph, this organs overwhelming innate and adaptive immune cells, and it plays important roles in host defense against the invasion of exogenous pathogens. Some studies have indicated that the liver is a lymphoid organ and that the immune response may initiate in the liver (8, 9). These studies hypothesize that the direct or indirect priming of lymphocytes is facilitated in this organs by the potential contact between circulating lymphocytes and antigens displayed by antigen-presenting cells in the sinusoids. The disease is characterized by nonspecific symptoms, including undulant fever, weight loss, depression, hepatomegaly, and splenomegaly. Arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis (10). This paper discusses well-characterized murine models of brucellosis that have been used to study infection and disease caused by *Brucella melitensiss.*

Materials and Methods

- **Animals and History:** The present study was carried out on a total number of 20 female mice (8 weeks of age) obtained from laboratory animals were divided into two groups. Group A: 10 mice injected with *Brucella melitensis* and group B: 10 mice without injected as a control. Mice were kept in conventional animal facilities and received water and food at liberty.
- **Isolation and Identification of *Brucella*:** *Brucella* was isolated from sheep abortion case and identification for Brucella according to the technique recommended by Alton, *et al*. (11). Bacteria was first grown onto Brucella agar under appropriate condition and was used for subsequent experimental infection of mice. Briefly, from Brucella agar, single colony of bacteria was transferred into 10 mL of Brucella broth and incubated at 37C^{o} for 72h. The concentration of bacteria in the broth was adjusted to 0.5 McFarland turbidity standards and from which 1 mL, approximately containing 5×108 cfu was used to infect the mice intraperitonially by the methods described previously by Zerva, *et al* (12). In addition, 10 mice, injected with 1 mL

of Brucella broth, and used as a negative control group. Samples (liver and spleen) were collected over a 6 weeks period of infection and kept in 10% formalin for histopathological study.

- **Sample collection:** After 6 weeks following exposure, 5 mice (group A) and two mice (group B as a control), liver and spleen were prepared for histopathological examination.
- **Histopathological Examination:** specimens included liver and spleen were collected and fixed in 10% formalin solution then washed, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness and stained with hematoxyline and eosin as a routine work for histopathological studies according to Bancroft & Stevens (13).

Results

- **Histopathological changes:** *Brucella* infection in the mice, the spleen is the most heavily colonized organ, and it showed mild hyperplastic activation of the white pulp with the presence of abundant histocytic and plasma cells around the medullary cords of the red pulp (Fig.1). Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell microgranuloma was also detected surrounded by the rem of lymphocytes and there are some of fibroblast cells (Fig. 2). The liver is also an important site for colonization and replication of Brucella in the mouse. Usually, mice infected with *Brucella* have mild to moderate hepatitis, which is characterized by neutrophils infiltrate at early stages of infection (Fig. 3), followed by histocytic infiltrate with epithelioid cells and microgranuloma lesions at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranuloma lesions (Fig. 4).

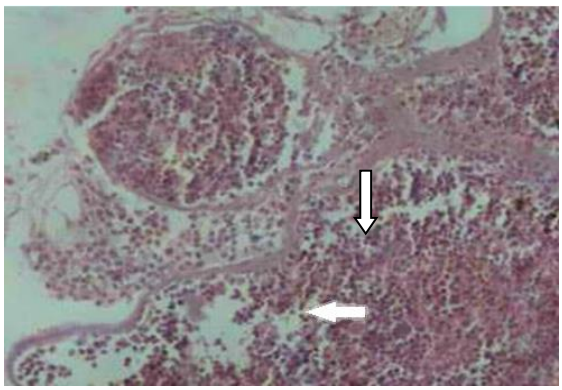

Fig (1) Spleen-histocytic and plasma cell around the medullary cord of the red pulp H & E. stain (15x)

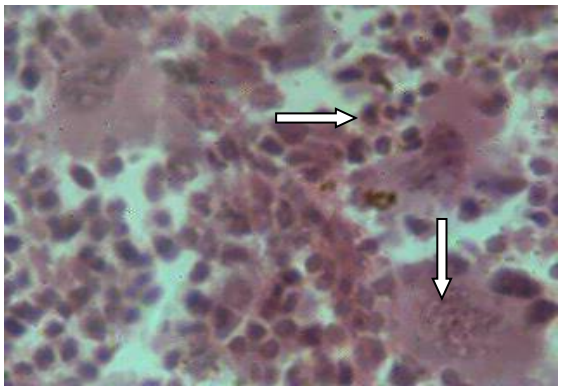

Fig (2) Spleen-Epithelioid and giant cell microgranuloma. H & E. stain (40X)

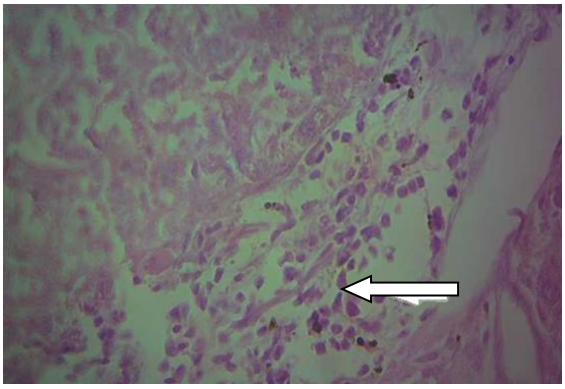

Fig (3) Liver- neutrophilic infiltration at early stages of infection. H & E. stain (40X)

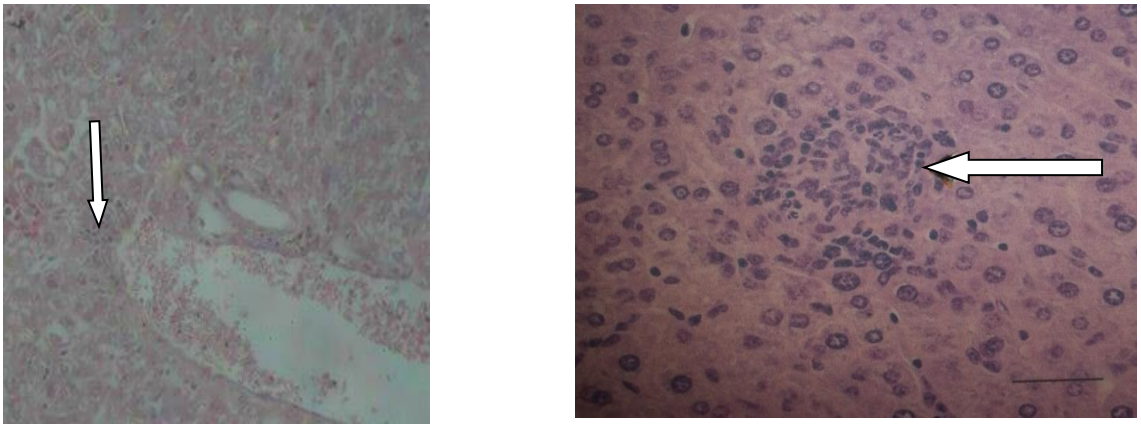

Fig (4) Liver- microgranuloma lesions. H&E. stain (40X)

Discussion

Brucella melitensis is the most invasive species and produces the most serious infection in human and animals (14). Analysis of the results reported here documents that intraperitonial exposure of mice to 105 cfu of *Brucella melitensis* leads to dose-related hepatic and splenic inflammation with persistence of bacteria in tissues of the mononuclear phagocyte system at least 6 weeks. During *Brucella melitensis* infection of the mice, the spleen is the most heavily colonized organ, and it develops hyperplastic activation of the white pulp with the presence of abundant histiocytes and plasma cells around the medullary cords of the red pulp (15, 16, 17) (Fig. 1). Mice intraperitoneally infected with *Brucella melitensis* develop significant proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell microgranuloma was also detected (17, 18) (Fig. 2). In another study conducted by our laboratory group, we reported that splenic white pulp to red pulp ratios increased in mice exposed intranasally to *Brucella melitensis*, similar findings in the study reported here require further characterization to ascertain the specific cell populations and subpopulations of the splenic white pulp responsible for the morphologic changes detected (19, 20). Also, investigation of quantitative changes in red and white pulp during the course of infection from onset to resolution may provide insights on the ability of *Brucella* to stimulate the host response (21). Histopathological examination revealed that hepatitis increased in severity with virulent strains of *Brucella melitensis* in mice. The liver is also an important site for colonization and replication of *Brucella melitensis* inside kuffers cells (16, 22, 23). which is characterized by neutrophilic infiltration at early stages of infection (Fig. 3), followed by histiocytic infiltration with epithelioid cells and microgranulomas at chronic stages of infection with bacteria localizing intracellularly in macrophages within microgranulomatous lesions (Fig. 4). The results reported here support this finding in that there was an apparent chronologic variation in lesions evident in the liver, some hepatic lesions were small and consisted of a few intrasinusoidal lymphocytes in contrast to other areas in which larger aggregates of lymphocytes and a mixture of histiocytes and neutrophils expanded and replaced hepatic architecture (15,17). This variation may reflect intermittent hepatic inoculation after periodic bacteremia. We were not surprised to see evidence of hepatitis because the liver is a documented target organ for humans and other animals with brucellosis. Hepatitis clinically manifested as an increase in activity of transaminases is evident in approximately half of the people with brucellosis, although substantial hepatic disease is evident in only a small percentage (24). It is noteworthy that *Brucella* infection in mice results in lesions that mimic those described in chronic infections in humans. Patients with chronic brucellosis may develop splenomegaly and hepatomegaly. Additionally,

multifocal granulomas with epithelioid macrophages are observed in the parenchyma of the liver and spleen in biopsy samples from infected patients (25, 26). However, hepatic and splenic abscess were described as uncommon complication in some patients during the acute phase of *Brucella sp*. infection (27). Brucella sp. chronic infection in humans may also lead to osteoarticular disease, including osteoarthritis, spondliytis, and osteomyelitis (1, 4). A previous study reported that mice may develop bacterial colonization in osteoarticular tissues during chronic stages of *Brucella melitensis* infection (28, 29). It is concluded that the histopathological changes in liver and spleen caused by *Brucella melitensis* in mice are similar to those observed in humans with brucellosis, the animal models, particularly the mice, have can be used and allowed for accumulation of valuable information of pathogenesis of *Brucella spp.* in vivo.

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