Histopathological changes in liver and spleen of mice infected with brucellosis

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Abstract:
A total of 20 female mice (8 week of age) a 10 mice control and 10 deal with 10⁵ cfu per animal of Brucella melitensis by injection intraperitonial. Samples were collected over a 6 week period of infection. Blood collected for serological test revealed positive for animals were injected with bacteria then animal dissected, liver and spleen collected and kept in 10% formalin for histological study. Results showed Histopathological changes show congestion, granulomatous, fatty change, fibrosis in liver and increased number of lymphohistiocytic cells and increased amount of white pulp. The study concluded that the pathological changes in liver and spleen caused by Brucella melitensis in mice are similar to those observed in humans with brucellosis.

Key word: Brucella, liver, spleen.

Introduction
Brucellosis is an infectious disease caused by bacteria of the genus Brucella that affect humans as well as domestic and wild animals, leading to significant impact on public health and animal industry. Brucella spp. is a Gram negative, facultative intracellular bacterium that is able to survive and replicate in phagocytic and nonphagocytic cell, establishing a chronic infection in both humans and animals (1). Human brucellosis is considered as a life-threatening debilitating disease characterized by weakness, fever, malaise, arthritis, osteomyelitis, endocarditics or meningoencephalitis(2) The disease is characterized by nonspecific symptoms, including undulantfever, weightloss, depression, hepatomegaly, splenomegaly, arthritis, spondylitis, osteomyelitis, epididymitis, and orchitis, as well as other more severe complications as neurobrucellosis, liver abscesses, and endocarditis, are also commonly described in patients(3,4). Brucellamelitensis, is one of the six species of Brucella, of which are known to be capable of infecting humans(5,6). The mouse has been the most widely used brucellosis model. Mice were first used by Holth in 1911 for Brucella vaccine testing. Thereafter, mice were used for etiological confirmation of samples from infected animals, to test virulence and for the evaluation of the pathological lesions(7). The results in mice are not immediately applicable and transferable to humans or to the target animal species. However, the uncovering of a significant phenotype in mice using an appropriate protocol gives useful information(8).

Brucellainfection may occur by digestive route, inhalation or through nasal mucosa or conjunctiva (9,10). After crossing the mucosal barrier, the organisms reach regional lymph nodes, replicate in macrophages, and establish a systemic and persistent infection. A bacteremia phase of infection results in colonization of the spleen, liver, and osteoarticular tissues, and depending on the Brucellaspecies and host, it may also colonize the mammary gland and the reproductive system (9,10,11).

In murine models of Brucella infection, experimental inoculation is performed mostly through three routes: intraperitoneal, digestive, or nasal (aerosol).

MATERIAL 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 distributed into two groups. Group A: 10 mice infected with B. melitensis and group B: 10 mice without infected as a control. Mice were kept in conventional animal facilities and received water and food ad libitum.

Isolation and Identification of Brucella: Brucella was isolated from sheep abortion state and identification for Brucella according to the technique recommended by Alton et al. (12). 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 37°C for 72h. The concentration of bacteria in the broth was adjusted to 0.5 McFarland turbidity standards and from which 1 mL, approximately containing $5 \times 10^8$ cfu was used to infect the mice intraperitoneally by the methods described previously Zerva et al. In addition, 10 mice, each injected with 1 mL of Brucella broth, and used as a negative control group. Samples were collected over a 3-4 week period of infection and kept in 10% formalin for histological study.

Sample collection: After 120 days following exposure, 5 mice (group A) and two mice (group B as a control), liver and spleen were taken from brucella seropositive cases for bacteriological. The organs are prepared for pathological examination.

Histopathological Examination: specimens included liver and spleen were collected and fixed in 10% formalin solution then wash, dehydrated, embedded in paraffin, sectioned at 4-5 micron thickness and stained with hematoxylin and eosin as a routine work for histopathological studies Bancroft & Stevens. RESULTS:

Histopathological changes
During Brucella infection in the mouse, 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 (Figure 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 microgranulomatous at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranulomatous lesions (Fig 4).
Fig (3) Liver neutrophil infiltration at early stages of infection. (40X)

Fig (4) Liver microgranulomatous lesions. (15X)

Discussion:

*Brucella melitensis* is the most invasive species and produces the most serious infection in human and animals\(^{(15)}\). Our study are revealed the histological changes in liver and spleen and the role of brucella in damage of these organs and others else. The histopathological changes in the liver revealed degenerative changes associated with focal leukocytes infiltration. Young et al.,\(^{(16)}\) reported that poorly formed hepatic granuloma, composed of leukocytes infiltration with or without necrosis, was demonstrated in mice infected with *Brucella melitensis*. The spleen showed mild hyperplastic activation of the white pulp with the presence of abundant histiocytes and plasma cells around the medullary cords of the red pulp. Active proliferation of reticulum cells was the characteristic picture in most cases. Epithelioid and giant cell granuloma was also detected. These results agree with El-Nesser et al.,\(^{(17)}\). Mice intraperitonially infected with *B. melitensis* develop significant splenomegaly, which is more prominent than in mice infected by aerosol\(^{(18,19)}\). During brucella sp. Infection in the mouse, the spleen is the most heavily colonized organ, and it develop histiocytes infiltrates and multifocal microgranuloma\(^{(20,21)}\).The liver is also an important site for colonization and replication of brucella sp. In the mouse,\(^{(22)}\) Mice infected with virulent strains of brucella sp. Have mild to moderate hepatitis, which is characterized by neutrophils infiltrate at early stage of infection, followed by histiocytes infiltrate with epithelioid cells and microgranuloma at chronic stages of infection with bacteria localizing intracellular in macrophages within microgranulomatous lesion\(^{(20)}\).In our study we are observed multifocal granulomas with epithelioid macrophages in the parenchyma of the liver and spleen in biopsy samples from infected patients that agree with Colmenero et al., and Akritidis et al.,\(^{(23,24)}\). The histopathological changes in the liver revealed degenerative changes associated with
focal leukocytes infiltration that agree with Young et al. reported that poorly formed hepatic granuloma, composed of leukocytes infiltration with or without necrosis, was infected with brucellamelitensis. Our study reported that the enlarged spleens and weight increased evident during necropsy were related to the brucella exposure that agreement with Mark et al. 

The study concluded that the pathological changes in liver and spleen caused by brucellamelitensis in mice are similar to those observed in humans with brucellosis. 

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Brucellamilitensis

دراسة التغيرات النسيجية في كبد وطحال الفئران المصابة ببكتريا Brucella:

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الخلاصة:

تناول البحث دراسة تأثير الإصابة ببكتريا البروسيلا من جنس Brucellamilitensis ودرجة الضرارة لها على أنسجة كلا من الكبد والطحال في الفئران. تضمنت الدراسة استخدام 20 من الفئران الإناث بعمر ثمانية أسابيع، 10 منها استخدمت كمجموعة سيطرة وأخرى تم تعتامها ببكتريا البروسلا حيث تم حقن $10^5$ من عزلة نقاء للبروسيلا في البروتون. حضنت البكتريا بالحيوانات لمدة ثماني أسابيع. بعدها تم تخدير الحيوانات وجمع عينات الدم لمفحوصات السيريولوجية ثم شرحت الحيوانات وجمعت الأعضاء المختبئة وتم تحضيرها في الدراسة النسيجية. أظهرت النتائج وجود تغيرات واضحة في أنسجة الكبد والطحال تمثلت بظهور احتقان وانماط وغيره في الكبد مع تغيرات وغيره في الكبد. وزيادة نسبة الليب الاحمر في الطحال مع تجمع للخلايا الالتهابية.