Study the ultrastructure changes in the liver of mice post infection with *Listeria monocytogenes*

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Summary

Listeria monocytogenes has important medical health infection in human and animals. The present study aimed to investigate the significant ultrastructure changes occurring in liver post-infection by pathogenic strain of *Listeria monocytogenes*. Twenty-six mice of both sexes were divided into two groups; 1st group (n=21) infected with 0.2 ml ($2x10^9$ CFU/ml) of *Listeria monocytogenes* I/P and 2nd group (n=5) served as negative control group; all animals of both groups were sacrificed at 2, 4, 6 and 24 hours post infection (7 mice for each time of infected group). The histopathology of liver tissues post infection revealed degenerative changes and severe necrosis in hepatic lobules exclusively at 6 and 24 hours. Also there were significant ultrastructure changes of hepatocytes; degeneration and necrosis of hepatocytes appeared at 2 hours post infection.

Keywords: *Listeria monocytogenes* infection, Transmission Electron Microscope, Mice, Liver.

Introduction

Listeriosis is an infection caused by *Listeria monocytogenes*, which is worldwide in distribution, and diagnosed more often in human in developed countries. Few people get listeriosis, but it is an important disease because of its high death rate due to brain inflammation (1) and blood infection. *L. monocytogenes* can colonize host cells and macrophages post bacterial invasion (2), then bacteria is able to escape from the phagosome by Listeriolysin O (LLO) and two phospholipase C enzymes (PLCs) and colonize the nutrient rich cytosol where they replicate rapidly (3 and 4). *Listeria* species have been isolated from various plant and animal food products associated with many listeriosis outbreaks; therefore, contaminated food are considered a primary source of transmission of infection in sporadic cases as well as outbreaks (5). These bacteria are well equipped to survive food processing technologies. For example they tolerate high concentrations of salt and relatively low pH and they are able to multiply at refrigeration temperatures. This makes listeria microorganisms a serious threat to food safety and ranks them among the microorganisms that most concern the food industry (6-8). The present study aimed to detect the pathological effects of *L. monocytogenes* on livers of mice at early stages of infection, based on the hisopathological and ultrastructural changes by using Transmission electron microscopy.

Materials and Methods

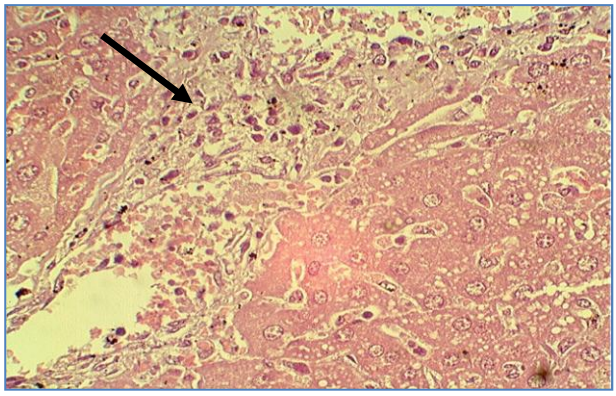
A virulent isolate of *L.monocytogenes* was obtained from the zoonotic diseases unit/ College of Veterinary Medicine. It was grown on brain-heart infusion broth and blood agar incubated at 37 °C for 24 hrs. and the bacterial counting was done (9) to determine the dose of injection (2×10^9 CFU/ml). Twenty-six white Balb/c mice (both sexes) were used in the present experiment and housed under optimal conditions, they were randomly divided into two groups as following: First group: (n=21) mice Infected I/P with 0.2 ml (2×10^9 CFU/ml) of *L. monocytogenes* bacterial suspension. Second group: (n=6) Injected with PBS 0.2 ml I/P as negative control group.

The animals of both groups were sacrificed at 2, 4, 6 and 24 hrs. post infection (n=7 for each subgroups) and pieces of their livers were preserved in 10% formalin for histopathological examination and other liver pieces for transmission electron microscopical examination were removed and cut into 0.5 mm^2 fragments and preserved in cold 1% buffered osmium tetroxide fixative at 4ºC for 4 hrs., followed by dehydration and embedding. Thin section was cut with an ultra-microtome with glass knives and picked up on uncoated 400-mesh copper grids then section was

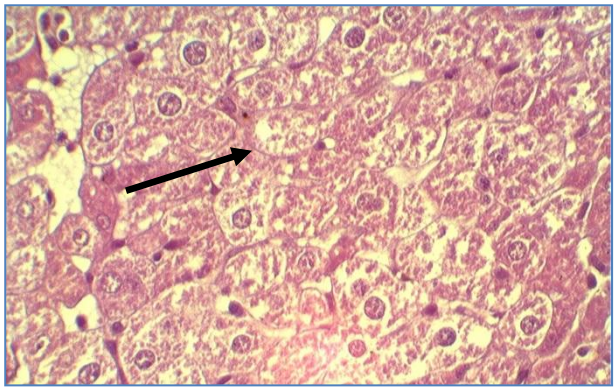
examined with a transmission electron microscope at College of Medicine-Al Nahrin University (10 and 11).

Results and Discussion

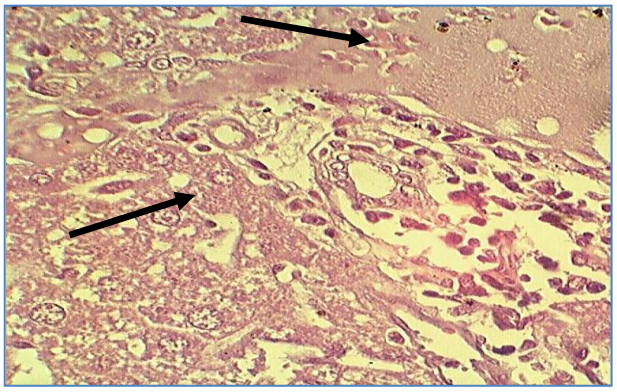
Two and four hours post infection; post-mortem examination showed hepatomegaly; enlarged and congested liver (dark color), mild to moderate splenomegaly, 6 and 24 hours post infection were normal. The hepatocytes were severely degenerated from acute cell swelling and hydropic degeneration which appeared swollen and enlarged, narrowing the sinusoidal cavities. Also there were single and small focal areas of coagulative necrosis with lyses of necrotic cells and presence of edematous fluid in dilated sinusoidal cavities also at 6 hours and increased at 24 hours post infection, the congested blood vessels contained inflammatory cells mainly polymorpho-neutrophils and few mononuclear cells (Fig. 1-4).



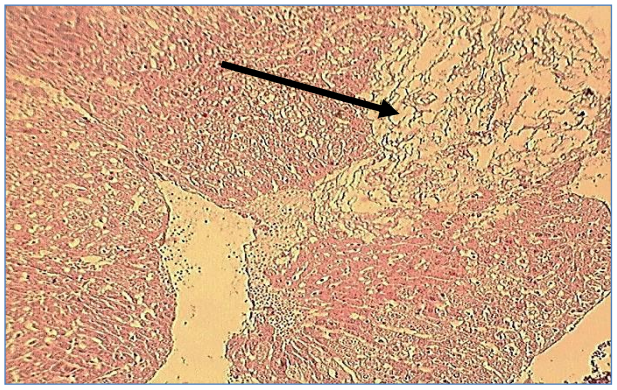
Figure, 1: Photomicrograph of liver 4 hours post infection; severe vacuolar degeneration of hepatocytes and infiltration of inflammatory cells in portal areas (arrow) (H and E stain, 400X).



Figure, 2: Photomicrograph of liver 6 hours post infection; necrosis of hepatocytes (granular appearance) (arrow) (H and E stain, 400X).

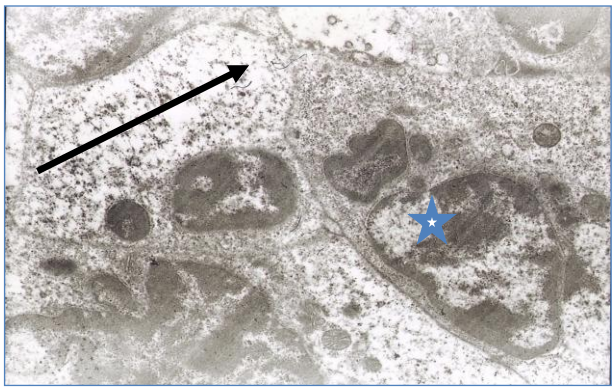


Figure, 3: Photomicrograph of liver 24 hours post infection; infiltration of inflammatory cells and accumulation of edema (arrow) in portal area (H and E stain, 400X).

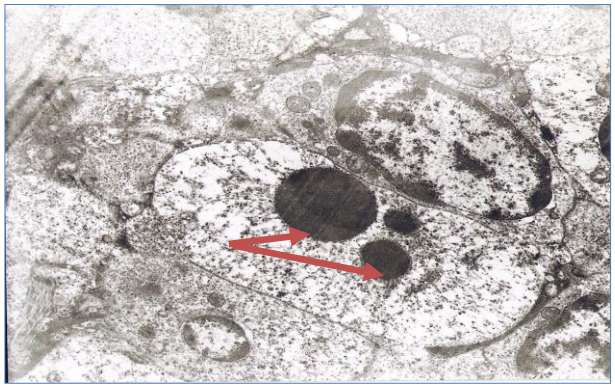


Figure, 4: Photomicrograph of liver 24 hours post infection; showed coagulative necrotic area of hepatic lobule (arrow) with atrophy of hepatic cords, dilated and filled with edematous fluid (H and E stain, 100X).

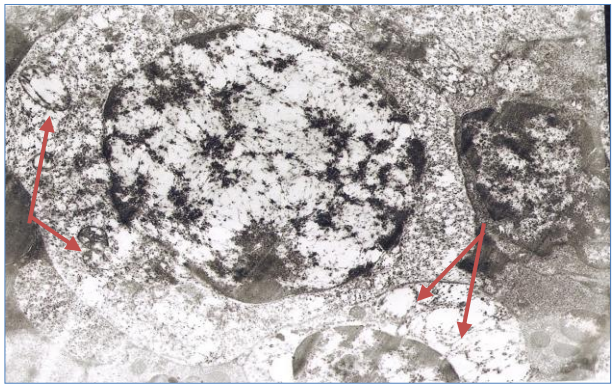
The histopathology of infected hepatocytes was compatible with results of electron microscopy. It showed variable changes at different times of infection; on periods 2 hours revealed the edematous smooth endoplasmic reticulum severely enlarged and dilated (Fig.5) with condensation of chromatin material at the nuclear membrane and vacuolation of mitochondria. The destructed chromatin material was seen separated from nuclear membrane (Fig.6), swelling of mitochondria and vacuolation (Fig.7). There was degeneration; elongated and enlarged mitochondria (Fig.8), destruction of cell membrane was seen; also there were many different micro-sizes of fat droplets with proliferation of lysosomes (Fig.9).



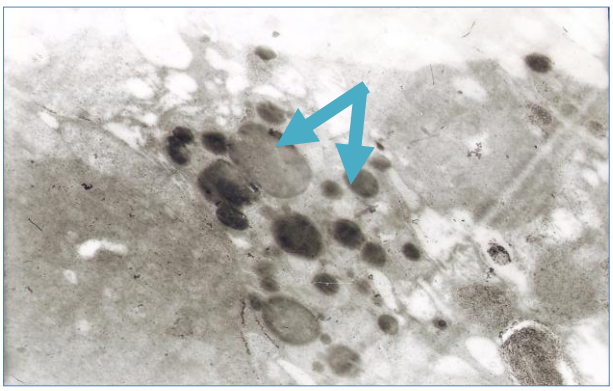
Figure, 5: Ultraphotomicrograph of Liver: 2 hours post infection: showed enlarged and dilated of edematous of endoplasmic reticulum (black arrow) with condensation of chromatin material (blue star) (70000).



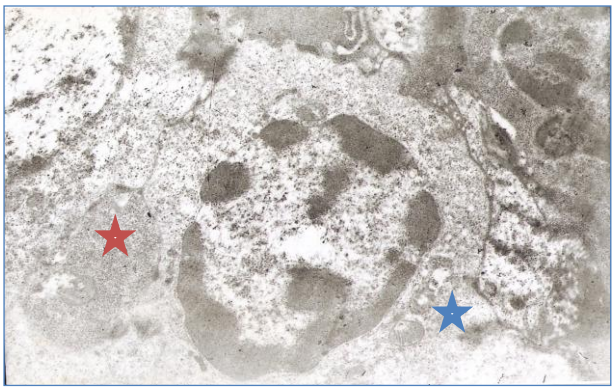
Figure, 6: Ultraphotomicrograph of Liver: 2 hours post infection; showed dark fat droplets (red arrows) (70000).



Figure, 7: Ultraphotomicroghraph of Liver: 6 hours post infection showed the swollen and vacuolated mitochondria (red arrows) with separated nuclear chromatin (>70000).



Figure, 8: Ultraphotomicroghraph of Liver: 6 hours post infection many fat droplets (blue arrows) (>70000).



Figure, 9: Ultraphotomicroghraph of Liver: 24 hours post infection with enlarged and vacuolar mitochondria (red and blue stars) with segmented nuclear chromatin (>70000).

The electron microscopy examination of infected liver revealed severe degenerative changes of hepatic cellular organelles which varied according to different post infection periods. It ranges from swelling and dilation of smooth endoplasmic reticulum at 2, 4 and 6 hours post infection which compatible with histopathological changes which showed severe vacuolar degeneration of hepatocytes and necrosis which agreed with other research it was explained that presence over 100-fold more Listeria than normal in the liver of mice depleted from polymorphoneutrophils by 24 hours of infection (12). Nuclear chromatin clumping and destruction of cell membrane mostly occurred at 24 hours post infection that may cause by the action of LLO, which is the most potent virulence factor of this intracellular pathogen, it is a pore-forming toxin responsible for the escape of the pathogen from the phagocytic vacuole into the cytosol of the host cell (4, 8, 13). It was demonstrated that localization of *Listeria monocytogenes* in the peribronchiolar space and invaded alveolar macrophages. Dissemination from the lung into the deep organs started almost immediately after application (2). The cellular pathological changes post infection in hepatocytes explained bacterial presence, but reduced bacterial numbers even absent from the cells, and due to host immune response by action of IFN-γ and IL-6. Both are important determinants cytokines in the protection of the epithelial cells from intracellular multiplication of *L. monocytogenes* (5 and 14-16). Many researchers proved that *Listeria* infection of hepatocytes respond to releasing

neutrophil chemotactants which kill bacteria, lyses infected host cells, detected in the present histopathology of hepatic tissues infiltrated with polymorphoneutrophils that caused liquefaction and lyses of hepatocytes (necrotic cells) few hours post infection, (6, 17 and 18); moreover they stimulate apoptotic process and secretion of IL-6 a cytokine suppress intracellular replication in hepatocytes and neutrophil-mediated defense mechanism can reduce >90% of bacterial growth in the liver during the first 24 hours of infection (19). Conclusively the significant pathological effects of *Listeria monocytogenes* on murine hepatocytes at different intervals post I / P infection were detected by both histopathology and electron microscopy.

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دراسة التغيرات المرضية فوق الخلوية في كبد الفئران بعد الاصابة ببكتريا *Listeria monocytogenes*

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الخلاصة

نظرا لاهمية الخمج بجراثيم *Listeria monocytogenes* في الانسان والحيوان صُممت التجربة الحالية لدراسة التغيرات المرضية النسجية والخلوية باستعمال المجهرالالكتروني بعد الإصابة بجرثومة *Listeria monocytogenes*. استعمل ستة وعشرون فأراً تم تقسيمها عشوائياً إلى مجموعتين:المجموعة الأولى ضمت واحداً وعشرين حيوانا حُقنت بمقدار 0.2 مل (2×10^9 خلية جرثومية/مل) بالخلب، أما المجموعة الثانية (خمسة فئران) حُقنت بالمحلول الملحي الفسلجي 0.2 مل كسيطرة سالبة. تمت التضحية بحيوانات التجربة لكلا المجموعتين بعد 2 و4 و6 و24 ساعة على التوالي، اظهرت نتائج الفحص النسجي لعينات الكبد المصابة تغيرات تنكسية وتنخر شديد خاصة للفترة 6 و24 ساعة بعد الخمج فضلاً عن التغيرات الشديدة في الخلايا الكبدية بوساطة المجهر الالكتروني خلال المدد المبكرة (2 ساعة) بعد الخمج والتي شملت نخر وتغيرات تنكسية حادة.

الكلمات المفتاحية: العدوى بالليستريا مونوسايتوجينز، المجهر الالكتروني النافذ، الفئران، الكبد.