Histpathological studies on BALB/c mice experimentally infected with *Leishmania major*[*]

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Abstract

The histopathological changes of the various organs (liver, spleen, kidney, heart, lung, ovary, testes) and body surface (footbad and skin above the base tail) of BALB/c mice Experimentally infected with *Leishmania major* were studies in details. The necrosis, heavily infllrated tissues with amastigotes, granuloma, inflammation of giant cells, hyperplasia, and thickening of glomerular. Basement membrane was among the main pathological changes noticed on mice during the present study.

دراسة نسيجية مرضية للفئران المختبرية نوع BAbl/C المصابة بطفيلي *Leishmania major*

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الخلاصة

درست التغيرات النسيجية المرضية على اعضاء مختلفة (كبد – طحال – كلية – قلب – رئة – مبايض وخصى) وكذلك على الجلد الخارجي (الاقدام الخلفية – الجلد اعلى الذنب) للفئران المختبرية نوع Balb/C اصيبت تجريبياً بطفيلي اللشمانيا ميجر.

تضمنت التغيرات النسيجية المرضية في الدراسة الحالية على الفئران المختبرية موت موضعي وارتشاح شديد للانسجة من قبل طور الامستجوت للطفيلي – ورم حبيبي التهاب – فرط تنسج وتثخن الغشاء القاعدي الكبيبي.

Part of M.Sc. Thesis

Introduction

Leishmanial infections induce both a humoral and a cellular immune response, and host recovery depends on the development of cellular immunity (Garcia and Bruckner, 1993). The infection with Cutaneous leshmaniasis causes histological change in the visceral organs with resembling visceral disease (Youssef, *et al.*, 1996).

The ulcer takes an a craterlike appearance, the thickened edges limiting its size, there is formation of granulomatous tissues, with eventual spontaneous healing. However, ulceration may leave large disfiguring scars (Garcia and Bruckner, 1993).

The histopathological changes that occur in simple CL are dominated by evidence of delayed (cell-mediated) hyper sensitivity to parasite antigens (WHO, 1990).

The nature of the inflammatory cells present in the smears was correlated with the number of *leishman donovani* bodies present (Dabiri, *et al.*, 1997).

Leishmania live exclusively in monocytic cells (monocytes, histiocytes and macrophages). Promastigote that do not penetrate monocytes are killed, probably by complement mediated lysis.

The aim of present study is to investigate the histopathological changes of skin and some internal organs of the experimentally infected mice.

Materials and methods

A- *Leishmania* strain and culture media

The strain of cutaneous leishmaniasis, which used in the present study was clinically identified as *Leishmania major*. This strain was isolated from female patient aged 35 years by specialized dermatologist Dr. Kathem K. Al-Rubiay.

The patient has multiple ulcerative lesions on her right arm and left thigh with a disease history of 3 months. The total number of lesions is

twelve (three on the side of the left thigh and nine on the right fore arm). The patient has not received any anti-leishmanial therapy.

Aspirate material from margin of ulcer was spread on a pre-cleaned slide. The prepared smear was stained with leishman's stain. Amastigote was seen under the light microscope. The *Leishmania* strain was isolated on diphasic Nicolle-Novy-MacNeal (NNN) medium.

The culture media were used during this study as follows:

The Nicolle-Nove-MacNeal (NNN) diphasic medium (Kagan and Norman, 1970; Meredith, *et al.*, 1995)was used for primary isolation of parasites from lesions aspirate of suspected C.L. and *in vitro* maintenance and sub-cultured of the *Leishmania* strain in addition to preparation of the injection doses and for treatment study *in vitro*.

The promastigotes were cultivated in diphasic media at (26-28)°C then harvested on the 6^{th} day either for animals infection or for sub-culturing in new media. The number of promastigotes per ml was determined by counting in hemocytometer and adjusted to $1x10^7$/ 0.1ml for inoculation.(Hazra, *et al.*, 1987).

BALB/c mice, 8-10 weeks old with a body weight of approximately 20-25 gm, were used in this study. Each mouse was inoculated with a dose $1x10^7$/ 0.1 ml of promastigotes at the hind footpad (each footpad received 0.05 ml) and with a dose $1x10^7$/ 0.1 ml promastigotes subcutaneously in a shaved area above the tail. Mice were used for histopathological studies after 14 weeks post-infection.

B- Histopathological study (Baker and Silverton, 1978):

1- Small portions (segments) of internal (liver, spleen, kidney, lung, ovary testis, heart) organs of mice were fixed in 10% formalin for 24hr.

2- They were dehydrated through graded ethyl alcohol as follows:

75% for ½ hr. , 80% for ½ hr., 85% for ½ hr., 90% for 1hr., 95% for 1 hr., 99% for overnight (24hr.).

3- The tissues were cleared in xylol for two hours and kept in mixture of xylol and molted paraffin wax at 60°C for two hours (3:1, 1:1, 1:3).

4- The tissues were embedded in paraffin wax in 60°C and later blocks were prepared and kept in refrigerator at 4°C and sections were cut at 5μ by the microtoms.

5- The sections were transferred to the water-bath at 40°C, fixed to glass slides and dried overnight in oven.

6- The slides were deparaffinized in xylol stained in Mayer's haematoxylin-Eosin stains.

7- Permanent mount were made in D.P.X.

Results

Histopathological changes of the various organs and body surface were studied in details as follows:

1- Liver: Microscopical examination of the liver tissue showed necrosis, heavily infiltrated of tissue with *Leishmania* amastigote form, Figures (1), (2). Pre-vascular granuloma and pre-vascular inflammatory infiltration were also seen. Figure (3).

2- Spleen: Microscopical examination showed numerous giant cells with multinucleate, Figure (4). Extensive chronic inflammation and heavy infiltrated with *Leishmania* amastigote Figure (5). Hyperplasia of the lymphoid follicles with a mild congestion of red pulp were also seen. Figure (6).

Amastigotes in histocytes of spleen tissue were observed, Figure (7).

3- Kidney: Thickening of glomerular basement membrane was seen Figure (8) with pre-vascular congestion Figure (9).

4- Footpad: Amastigotes were numerous throughout the infected skin dermis. Figure (10). The epidermis contains large number of macrophages with necrosis and inflammatory surrounding area. Figure (11). Leishmanial bodies was noticed in histocytes of the dermis of footpad skin. Figure (12).

5- Skin ulcer above the base tail: During this study it was found that the ulcered skin showing dermis with extensive inflammation with neutrophils and plasma cells, Figures (13) and (14).

6- Heart, Lung, Ovary and Testes: Microscopical examination showed no pathological changes in these organs.

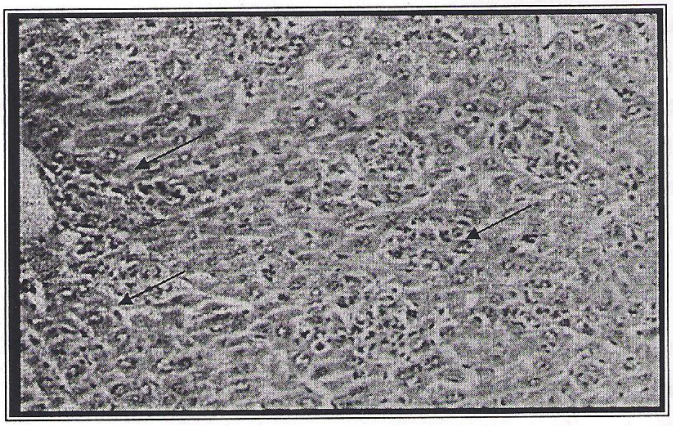

Fig. (1): Liver of infected mouse showing necrosis and heavily infiltrated with *L.major* amastigote (89X).

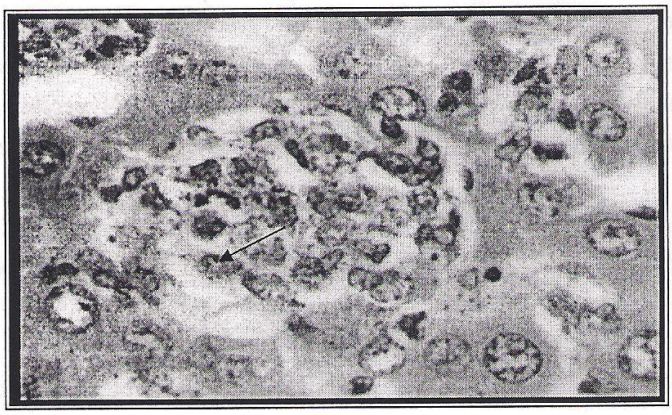

Fig. (2): Liver of infected mouse showing numerous extracellular and intracellular of *L.major* amastigote (359X).

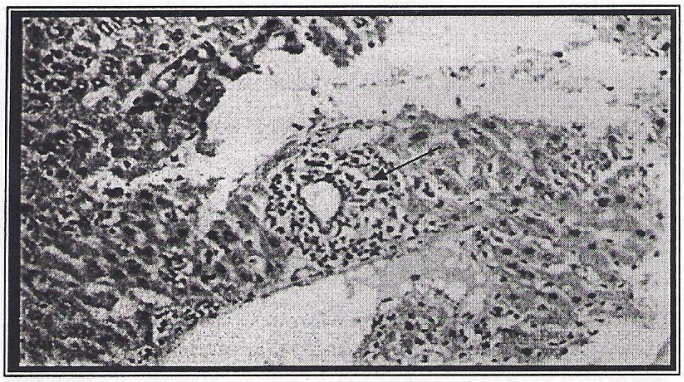

Fig. (3): Liver of infected mouse showing perivascular granuloma and inflammatory response (89X).

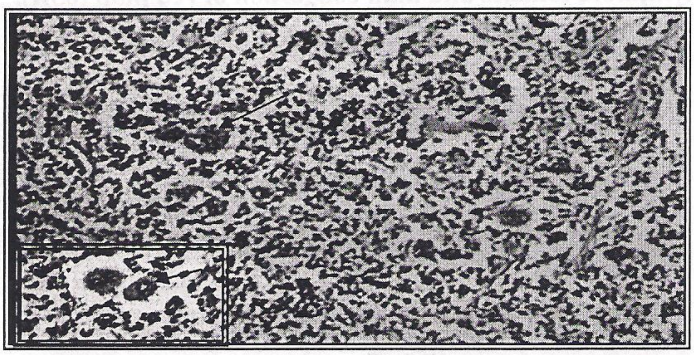

Fig. (4): Spleen of infected mouse showing frequent giant cells (89X) (inserted figure showing enlarged giant cells (359X0).

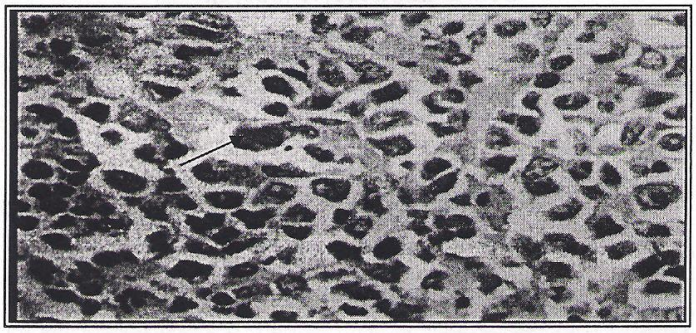

Fig. (5): Spleen of infected mouse showing numerous amastigote of *L.major* with diffuse macrophage (359X).

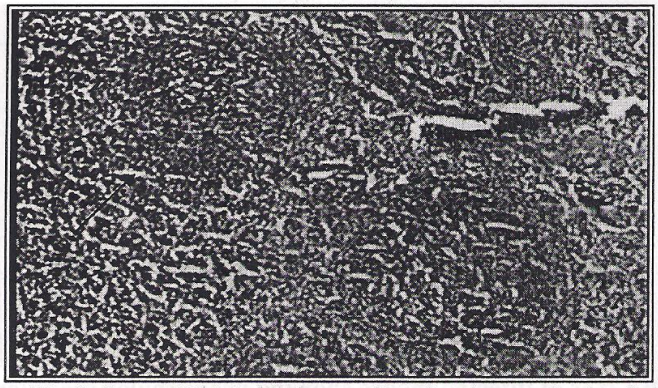

Fig. (6): Spleen of infected mouse showing hyperplasia of the lymphoid follicles and mild congestion of red pulp (63X).

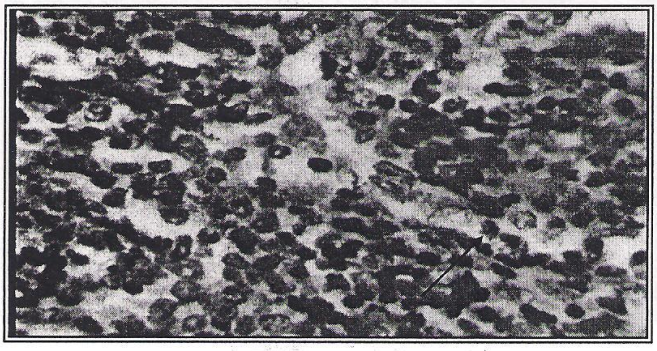

Fig. (7): Spleen of infected mouse showing leishmanial bodies in histocyte (359X).

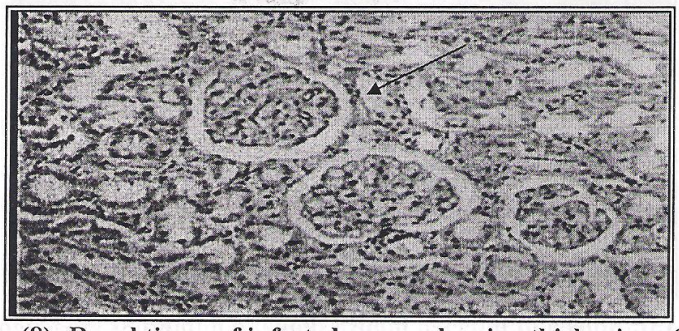

Fig. (8): Renal tissue of infected mouse showing thickening of glomerular basement membrane (513X).

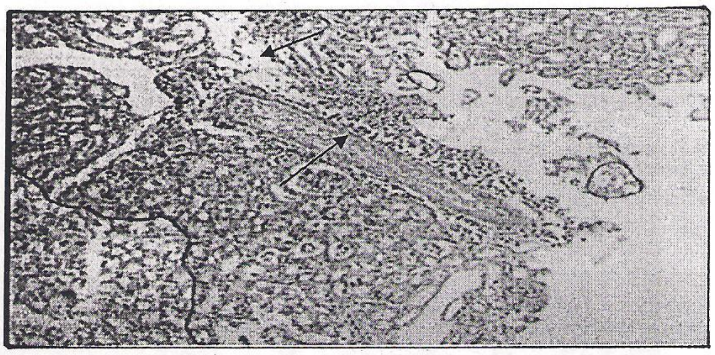

Fig. (9): Renal tissue of infected mouse showing prevascular congestion (128X)

Fig. (10): Footpad of infected mouse showing numerous *L.major* amastigote with macrophage in epidermis (359X).

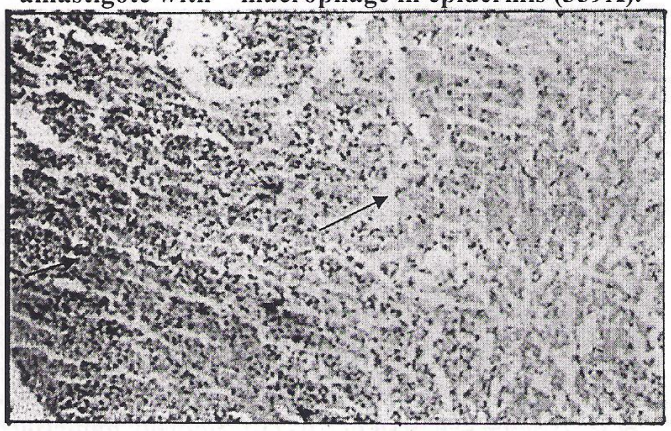

Fig. (11): Footpad of infected mouse showing necrosis and inflammatory area (89X).

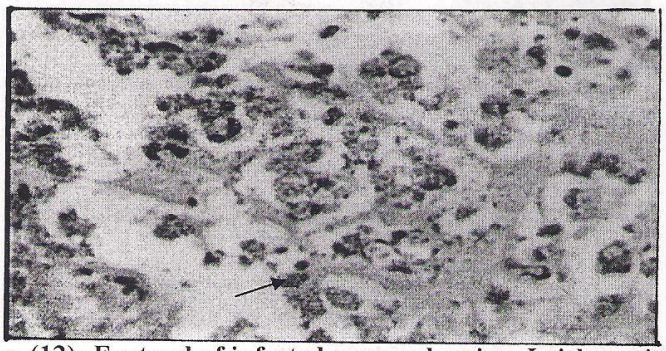

Fig. (12): Footpad of infected mouse showing Leishmanial bodies in histocyte (359X).

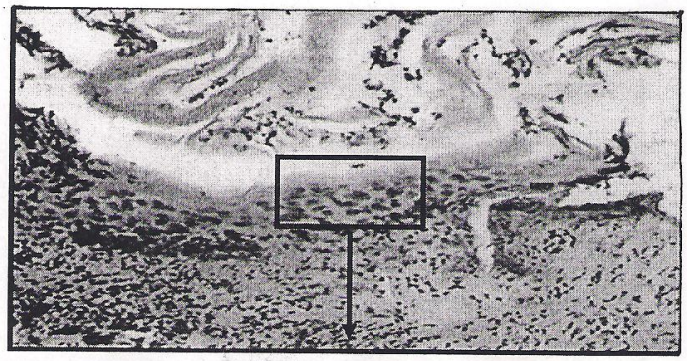

Fig. (13): Mouse skin ulcer at the tail base showing dermis with extensive inflammation with neutrophils and plasma cells (136X).

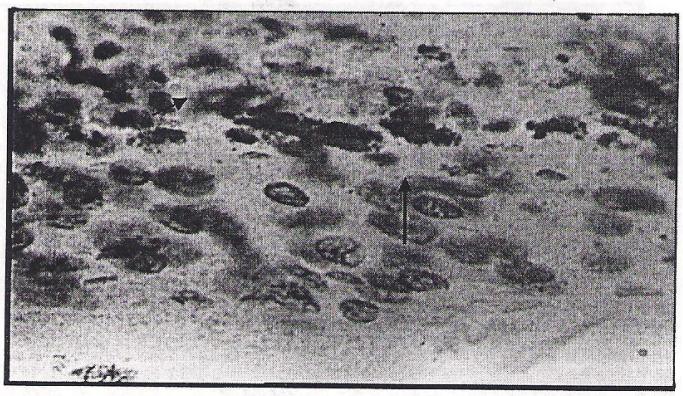

Fig. (14): Enlarged area in Fig. (13) showing numerous amastigote of *L.major* (542X).

Discussion

In the final host, *Leishmania* occurs as intracellular multiply in macrophages and other phagocytic cell of the reticuloendothelial system. The pathological changes that characterized the various clinical forms of the disease reflect the balance between parasite multiplication, the immune response and resultant degenerative changes (WHO, 1990).

The histopathological characters of liver of infected mice showed necrosis and inflammatory response at the 12th week post infection. The granuloma was among the main histopathological changes in the liver.

Previous studies have shown that granuloma was occurred in mice experimentally infected intradermaly with *L.major* (Abbel-Wahab, *et al.*, 1988) . On the other hand, liver granuloma formation, diffuse inflammation and the liver infiltration with amastigote was noticed in swiss albino mice inoculated with Saudi Arabian cutaneous strains of *L.tropica* (Youssef, *et al.*, 1996). The occurrence of granuloma in the liver was reported in BALB/c mice experimentally infected for 6 months with *L.donovani* (Cutierrez, *et al.*, 1984) . In BALB/c mice infected with *L.tropica* no histopathological changes was reported in liver (Scott and Farrell, 1982).

In the present study, the spleen of infected mice showed marked hyperplasia of the lymphoid follicles and congestion of the red pulp. The spleen was heavily infiltrated with *L.major* amastigotes, and showed many gaint cell with chronic inflammatory. The results of this study were coincided with the results that obtained by other workers using CL strain (Youssef, *et al.*, 1996). Similar findings were obtained in mice experimentally infected with *L.major* (Abbel-Wahab, *et al.*, 1988).

During the present study, the kidney of infected mice showed thickening in it's glomercular basement membrane and prevascular congestion. No invasion of the lung by *L.major* amastigote was observed. On the other hand, golden hamsters experimentally infected with *L.donovani* showed interstitial pneumonitis with septal fibrosis (Durate and Corbett, 1984).

No pathological changes and invasion of mice testes by *L.major* strain were observed in this study. These findings were also confirmed by other studies (Youssef, *et al.*, 1996). No pathological changes in heart and ovary were observed, and there is no report described the histopathological changes of these organs.

The histopathological study include the cutaneous of local site of inoculation in footpad and the area above the base of the tail. The cutaneous (skin) tissue dermis showed numerous amastigote in infected skin and histocyte of the dermis. Other studies have described inflammatory and the presence of amastiogote in dermis of skin in experimental animals infected with *L.tropica* (Eissa and Younis, 1997; Youssef, *et al.*, 1994)). It was found that the BALB/c mice are a good and suitable animal model host for studying the dynamics and histopathological changes of *L.major*.

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