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Original Research Article

Pathological Effects of *Leishmania Donovani* Promastigotes on Liver and Spleen of Experimentally Infected BALB/C Mice

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Abstract

Visceral leishmaniasis (VL) disease is recognized as an important public health problem in countryside (Iraq). The *Leishmania donovani* complex parasites can parasitize the reticuloendothelial cells**,** The parasite invades internal organs (spleen, liver, bone marrow). The effect of visceral disease of *Leishmania* parasites was studied on liver and spleen in BALB/c mice experimentally infected with *Leishmania donovani* promastigotes. *Leishmania donovani* (MHOM/IQ/1982/BCR1/AA3), were obtained from the *Leishmania* unit at medical research center of Al-Nahrian University. Cultures were carried out using Nicolle-Novy-McNeal (NNN) medium. A solid and liquid phase. The pathological effect was observed on two organs. The progressive of visceral infection was monitoring every two weeks for evaluation the development of weight and length of liver and spleen respectively. The results of this study have demonstrated that the infected mice shown the hepatosplenomegaly sign of pathological efficacy of *L. donovani* promastigote of mice inoculation. The weight of liver was increased with increasing days of infection until reached to (2.30) gm compared with control (1.60) gm of inoculated mice on 12 weeks after parasitic inoculation, also the length of spleen increased with increasing days of infection until reached to (40) mm compared with control (19) mm. Statistical analysis data of weight and length was highly significant differences ($p < 0.05$) between infected and control groups. It was concluded that the pathological changes in visceral organs liver and spleen in experimentally infected mice may be used as indicators for progression of the infection with its severity.

Key words: Visceral leishmaniasis, *Leishmania donovani*, pathological effect, BALA/c mice

التأثيرات المرضية للطور المسوط لطفيلي *Leishmania donovani* على الكبد والطحال للفئران المختبرية سلالة BALB/c المصابة تجريبياً

الخلاصة

يعتبر داء اللشمانيا الحشوي من المشاكل الصحية المهمة في المناطق الريفية (العراق). تتطفل كائنات اللشمانيا *Leishmania donovani* في الخلايا الشبكية الطلائية ، الطفيلي يهاجم الاعضاء الداخلية (طحال ، كبد ، نخاع العظم). تم دراسة تأثير المرض الحشوي لطفيلي اللشمانيا *Leishmania* على الكبد والطحال في الفئران المختبرية سلالة BALB/c mice والمحقونة تجريبيا بالطور المسوط لطفيلي *L. donovani* . تم الحصول على عزلة *L. donovani* (MHOM/IQ/1982/BCR1/AA3) من وحدة اللشمانيا في مركز البحوث الطبية لجامعة النهرين. تم توزيع الطفيلي بأستخدام الوسط الزرعي ثنائي الطور (NNN) Nicolle–Novy–McNeal المتكون من الطور الصلب والطور السائل. تم ظهور التأثير الأمراضي على اثنين من الأعضاء. تم مراقبة تزايد الأصابة الحشوية كل اسبوعين لحساب التطور الحاصل في وزن وطول الكبد للتأثير تباعاً. أثبتت نتائج الدراسة الحالية ظهور علامة تضخم الكبد والطحال للتأثير الأمراضي للطور المسوط لطفيلي *L. donovani* على الفئران المحقونة. وزن الكبد كان يزداد بأزدياد أيام الأصابة حتى بلوغه (2.30) غم مقارنة بمجموعة السيطرة (1.60) غم للفئران المحقونة عند الإسبوع ١٢ بعد حقن الطفيلي. ايضاً طول الطحال كان يزداد بأزدياد أيام الإصابة حتى بلوغه (40) ملم مقارنة بمجموعة السيطرة (19) ملم. إحصائياً يوجد فرق معنوي عالي ($p < 0.05$) لبيانات الوزن والطول بين المجاميع المصابة ومجاميع السيطرة . تم الإستنتاج من الدراسة الحالية بأن التغيرات الإمراضية في الأعضاء الحشوية ، الكبد والطحال تجريبياً ممكن ان تستعمل كدليل لتزايد الإصابة وشدتها.

الكلمات المفتاحية: داء اللشمانيا الحشوي ، لشمانيا دونوفاني ، التأثير الإمراضي ، الفئران المختبرية سلالة BALB/c .

Introduction

Visceral leishmaniasis or Kala-azar is still a common parasitic infection among children in Iraq [1, 2]. It is an endemic disease. Visceral leishmaniasis caused by geographical variants of the *Leishmania donovani* complex (*L. donovani, L. infantum, L. chagasi*), it is a progressive wasting disease of dog and humans that is often fatal if untreated [3, 4]. Visceral leishmaniasis is a major public health problem in the world, it is recognized as an important public health problem in Iraq and the most fatal form, classically which is, known as Kala-azar or black fever [5]. Visceral leishmaniasis transmitted chiefly by sandflies of the genera *Phlebotomus* (old world) and *Lutzomyia* (new world) [1, 2, 6] which are the only insects showing cyclic development of the parasites [7]. VL (infant kala-azar) typically affects children young than 5 years of age [8] the incubation period is variable but usually 2-8 month [9, 8], it may exceed 12 years [1]. The onset of clinical signs may be sudden, with acute manifestations, but in the usual cases it is insidious. On the average about 90 days following the exposure [10]. The aim of this study is to demonstrat and illustrat the pathological effect of *L. donovani* promastigotes in experimentally infected BALB/c mice.

Materials and Methods:

Leishmania donovani (MHOM/IQ/ 1982/BCR1/AA3), were obtained from the *Leishmania* unit at medical research center, Al-Nahrian University. Cultures were carried out using Nicolle-Novy-McNeal (NNN) medium. a solid and liquid phase [11, 12]. The parasite was cultured in a biphasic NNN medium containing 50 µg/ml of Gentamycin to check the parasite growth. The growth of parasites was examined after two days to ensure a good growth of the parasite and sterility. Liquid medium was added when needed in order to get the active parasites at the log-phase [13]. The promastigote parasites were harvested on the 6^{th} day after a fresh smear from liquid phase of biphasic culture was prepared and observed under light microscope. The number of parasites were adjusted to 1×10^5 /0.1 ml for mice inoculation by counting in hemocytometer. The expansions provided were examined under light microscopy after staining with Giemsa. Mice were divided into infected and control groups as follow both sex of BALB/c- mice aged 8-10 weeks, weighing 20-25g were obtained from the animal house of the college of science, Babylon University. The animals were fed with standard food and under standard condition for housing. The prepared impression smears were stained by Giemsa's stain to detect the Leishman bodies under the oil immersion objective of the light microscope every two weeks of infection. Infected mice and control were killed with ether and dissected every two weeks to monitor and evaluate the development of weight and length of liver and spleen respectively. Each value in the experiment was expressed as mean of three replicate. One way analysis of variance and least significant differences were used in the present study [14].

Results

The results of this study demonstrated that the weight of liver and length of spleen were increased with increasing the time of infection. The mean of weight and length for liver and spleen for infected mice was shown in Table (1, 2). Statistical analysis data of weight and length was with high significant differences ($p < 0.05$) between infected and control groups.

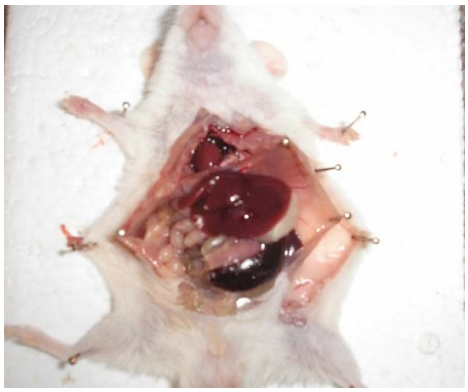
Figure (1) illustrated the picture of hepatomegaly and splenomegaly on 12 weeks of infected mice. The density of *L. donovani* amastigotes in liver and spleen for infected mice on 12 weeks are shown in Figure (2).

Table 1 : Mean of liver weight in control and infected groups of infected mice

Weeks after parasitic inoculation	Mean of liver weight (gm)	Control
2	1.05	0.9
4	1.20	1.0
6	1.55	1.2
8	1.95	1.3
10	2.10	1.5
12	2.30	1.6

Table 2 : Mean of spleen length in control and infected groups of infected mice

Weeks after parasitic inoculation	Mean of spleen length (mm)	Control
2	12	9
4	15	10
6	20	12
8	27	15
10	33	16
12	40	19



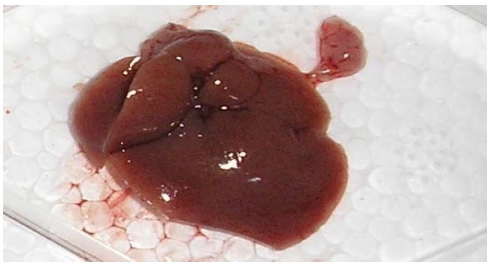


Figure 1 : A, Dissecting of BALB/c mouse; B, liver of mouse with weight (2.30 gm); C, spleen of mouse with length (40 mm) on 12 weeks after infection

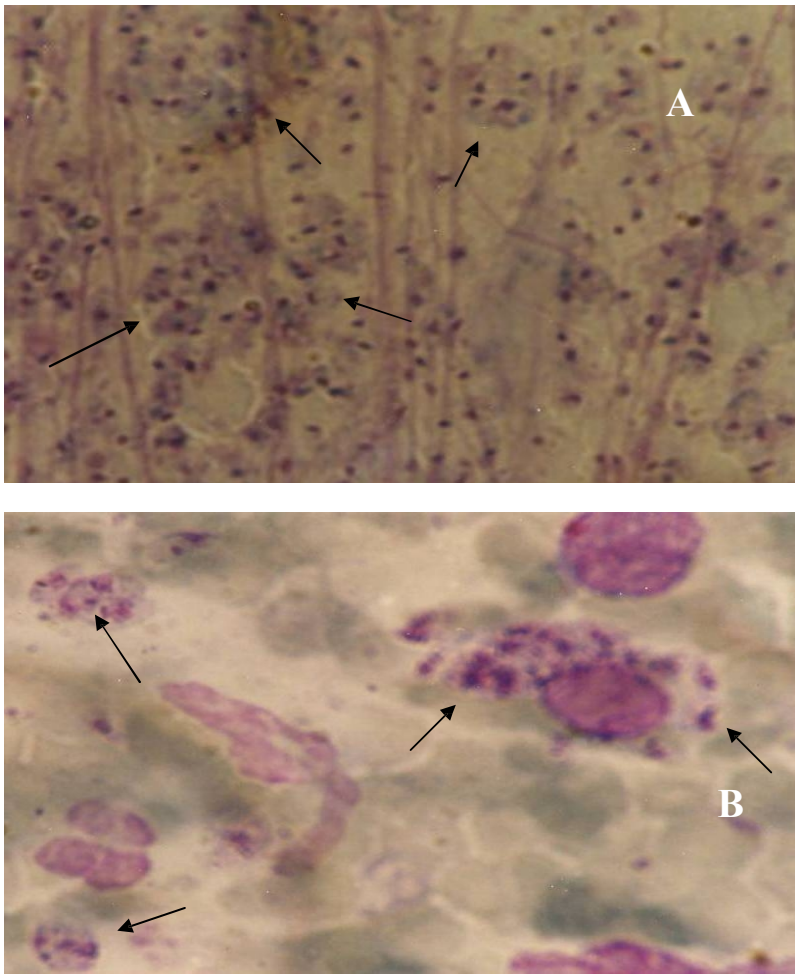


Figure 2 : A, Impression smear of mouse liver on 12 weeks after infection. B, impression smear of mouse spleen on 12 weeks after infection. showing dissemination of *L. donovani* amastigotes inside and outside macrophages

Discussion

The normal habitat of *Leishmania donovani* in man is the mononuclear phagocyte system (spleen, liver, bone marrow, intestinal mucosa and mesenteric lymph nodes). This parasite may be found in endothelial cells of the kidneys, suprarenal capsules, lungs, meninges and in cerebrospinal fluid [6]. Diagnosis of VL can be based on geographical presence of parasite, history of sandfly bite and clinical features, but does require considerable expertise [15]. The standard diagnosis of VL is parasite (amastigotes) identification in tissue smear, with spleenic aspirate, bone marrow and lymph node aspirate and staining by giemsa, although there are difficulties in obtaining and examining tissues [16]. Culture the aspirated microscopy-negative tissue samples on the NNN media or inoculated into experimental animals such as hamsters to study the parasite, or diagnosed the parasite by the isoenzyme analysis. The parasitological smear and culture examination showed presence of parasites in all experimental days in spleen and liver, the parasite had the ability to disseminate to visceral organs firstly to spleen at 4 weeks post infection liver [17]. The results of this study have demonstrated that the infected mice shows the hepatosplenomegaly sign of pathological effect of *L. donovani* promastigote in the infected mice. The weight of liver was increased with increasing days of infection until reached to (2.30) gm compared with control (1.60) gm of inoculated mice on 12 weeks after

parasitic inoculation, also the length of spleen increased with increasing days of infection until reached to (40) mm compared with control (19) mm. The parasites invade macrophages throughout the reticuloendothelial system and cause severs systemic disease with hepatomegaly, lymphadenopathy, pancytopenia, fever, and weight loss. The spleen may weigh as much as 3 kg and lymph nodes may measure 5cm in diameter (18). In this study the terminal stages of visceral infection the hepatosplenomegaly is massive, both the liver and spleen can be palpated far below the costal margin [10, 8]. Hepatomegaly and spleenomegaly, hall marks of progressive result from reticuloendothelial cell hyperplasia [19]. Jaundice is occasionally present in VL. Epistaxis, gingival bleeding and petechiae on the extremities of the patient appeared [15]. pancytopenia-anemia, leucopenia, neutropenia, marked eosinopenia**.** In the interaction between macrophage and parasite, there are various tricks for parasite survival within the macrophage [7]. In this study, microscopical examination of stained impression smear of liver and spleen showed the density of amastigotes in two organs that demonstrated the pathological effect of parasite.

The disease is reticuloendotheliosis, due to invasion of the reticular syncytium by leishmania. Free histocytes are gradually formed and also become parasitized. Late in the infection, the lymphatic reticulum of the spleen becomes involved. Microscopically, a marked increase in parasitized reticuloendothelil cells is seen. [6].

The results of progress infection virulence in inoculated of visceral mice were clear because the culture and impression smear of liver and spleen were positive during the period of infection.

In this study the parasites in spleen impression smear were shown clearer or faster than liver impression smear, that may because the spleen was regarded the main organs of peripheral lymphatic system, which have main function filtrated blood from harmful antigens because have large number of macrophages these macrophages are host of parasites in mammals, or that may be due to the difficulty finding parasites in liver because large size of it when compared with spleen, that is agreement with Ott *et al* [20] who demonstrated that spleen was more sensitive for infection when compared with liver. Moreover the number of parasites in spleen was more than for same period of inoculation. The spleen shows various degrees of enlargement from a barely palpable size to one that extends well below the level of the umbilicus. Enlargement takes place rapidly, a maximal size sometimes being obtained within three month [7, 6]. It becomes congested, purple or brown in color [6]. The liver is less frequently enlarged than the spleen. Although hepatic enlargement without spleno-megaly has been reported. The liver usually reaches maximal size by sixth months following the infection [7]. In the enlarged liver, there is fatty infiltration of Kupffer's cells and the endothelial cells of the blood vessels are invaded by amastigotes [6].

With the time, some of the parasitized, macrophages are set free into the blood stream or lymphatic and carried from the skin to the viscera, where they lodge and the parasites multiply rapidly, in the spleen, liver, bone marrow and other centers of mononuclear phagocyte system, the amastigotes are now taken up by fixed macrophages, such as Kupffer,s cells in the liver, multiply in these cells and destroy them [6, 10, 2]. These pathological lesions depend upon the virulence of the strain and the resistance of the individuals [7].

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