J.Thi-Qar Sci. Vol.3 (4) Feb./2013

ISSN 1991- 8690 الترقيم الدولي ٨٦٩٠ ـ ١٩٩١

website:http://jsci.utq.edu.iq Email:utjsci@utq.edu.iq

The experimental study of pathological changes of visceral leishmaniasis in dog in Basrah province

Dawood Salman Mahdi[1] Mahdi Murshd Thauiny[2]

[1]Health and Medical Technical College – Basrah [2] Nursing College- Uni. Of Thi-qar

Abstract

The aim of this study was to evaluate the histopathological effect of leishmaniasis on liver and spleen of dog. Five dogs were inoculated with leishmania in laboratory to study variable histopathological change in relation to serological analysis. Section of liver and spleen were cut and stained with Leishman and haematoxylin and eosin stain for microscopical examination. Liver was show a pseudolobule, few inflammatory cells infiltration mostly lymphocytes and plasma cells and liver parenchyma is absent with a few necrotic hepatocytes with areas of fibrosis. Spleen was greatly enlarged, section of it shown few sinusoids have large numbers follicular structures,eosinophilic hyalinized wall of blood vessels ,multiple areas of necrosis and destroying the normal architecture white pulp, red pulp is largely occupied by collection of macrophages (histiocytes). When compared with natural dog that have negative serological test.

الخلاصة

تضمنت الدراسة الحالية تقيم التأثيرات ألمرضيه ألنسيجيه في كبد وطحال الكلاب ألمصابه تجريبيا بطفيلي الليشمانيا. خمسة كلاب تم حقنها بهذا الطفيلي مختبريا لرؤية مختلف التأثيرات بعد الاختبار المصلي الموجب عند مقارنتها بالكلاب ذات الاختبار المصلي السالب. أخذت مقاطع نسيجيه من الكبد والطحال، صبغت بصبغة الليشيمانيا إضافة إلى الايوسين والهيماتوكسلين للفحص النسيجي ألمجهري. شوهد في عدد من المقاطع ظهور الكبد على شكل فصيصات كاذبة, وارتشاح عدد قليل من الخلايا الالتهابية في الغالب اللمفوسايت والبلازما مع غياب شبه كامل لمتن النسيج الكبدي، إضافة إلى وجود مناطق تنخريه وليفينيه. أظهرت عدد من المقاطع في الطحال حزم من نسيج ضام ليفيني بكميات مهمة, إضافة إلى نسيج زجاجي حمضي في جدار الاوعيه ألدمويه, ومناطق متعددة من التنخر مع تحطم لمتن النسيج الطبيعي في اللب الأبيض والأحمر من الطحال، كما لوحظ ارتشاح عدد كبير من الخلايا البلعميه (الهستيوسايت).

Introduction

Leishmania species are wide spread in the world, causing coetaneous and visceral infections in humans. The leishmaniasis appear to be far more abundant and of greater public health importance than was previously recognized with epidemics of both leishmaniasis occurring [1]. Most forms of the disease are transmissible only from animals (zoonosis), but some can be spread between humans. Human infection is caused by about 21 of 30 species that infect mammals. The dog is considered to be the primary domestic reservoir of the disease [2].

Visceral leihmaniasis is a chronic illness that is characterized by irregular fever, hepatosplenomegaly, anaemia and leucopenia, and progressive weakness and emaciation which can result in death if left untreated. The vector is female sandfly, and in most regions, the canine has been identified as the major reservoir for transmission [3]. The causative organism was first isolated in 1903. In vertebrates, the parasites are found intracellularly in the reticuloendothelial system as the amastigote form, which is flagellated, round, and 2-4 µm in diameter [4]. Most infected humans [3,5] and at least a proportion of infected dogs [5,6] remain asymptomatic or develop a mild disease, which is spontaneously cured. Occasionally, dogs and humans develop a severe form of VL [3,7] that is usually lethal if left untreated. In both humans and dogs, the disease proceeds with emaciation, enlargement of the liver and spleen, fever, anaemia, and an increased predisposition to bacterial infection [3,8–9]. In dogs, such signs of disease are further accompanied by a variety of skin and ocular lesions [10]. Much that is known about the immune response to Leishmania parasites [11].

In order to evaluate the role of the dog in the cycle of canine visceral leishmaniasis (CVL) infection, all manifestations of the disease must be known, ranging from clinical and pathological features to the extension and progression of lesions in various compromised organs. So we decided to examine the spleen because it play a central role in visceral leishmaniasis, in contrast liver.

Materials and methods

The animals were brought to the laboratory from Basrah streets after breeding and inoculated with leishmania (promastigote) via leg veins and lymph nodes. After two to three months we examine the clinical signs of leishminiasis. The presence of anti-leishmania antibodies in the serum were investigated by DAT [Direct Agglutination Test] (have positive test).

The animals were killed as recommended by the program for the control of zoonotic diseases that have clinical signs were obtained and records. Liver and spleen specimens were collected from five dogs that infected with leishmanial promastigote in libratory. As a control group, specimens from 3 animals with a negative LST, and the absence of anti-*Leishmania* antibody activity in the serum as measured by DAT were randomly selected; we referred to this group as non infected pathological examined.

Three to four mm-thick slices of liver and spleen tissue were cut transversally and fixed in 10% formalin, tissue slices were embedded in paraffin. Four to five µm-thick section were cut and stained with Leishman and haematoxylin and eosin stain for microscopical investigation. A section from liver and spleen of control group was taken. All tissues were processes by conventional methods. These sections were examined by the authors who were blind to previous knowledge of the identities of the animals.

Results

Clinical diagnosis:- temperature of undulant type of fluctuated daily from 36.6C to 39C, frequently with a double rise of temperature every 24 hours. The abdomen is a protuberant and both the liver and spleen can be palpated far below the costal margin, bleeding typically occurs in subcutaneous region of ventricle part of chest.

Cross lesions: the liver of dog which is exposed to experiment inoculates with promastigote of leishmania appear areas of consolidation with central necrosis and hemorrhage, also it appear as micronodula and hard, liver small enlarged and shrunken, firm but somewhat friable.

Microscopic lesions by histopathological sections in the liver was show apseudolobule, that is without a central vein and peripheral portal trials (fig. 1), with diffuse hydropic degeneration almost 1-2 section appeared the hepatocytes are vacuolated. Parts if section exhibited the liver is divided into more or less uniform sized pseudolobules, disruption of the architecture of the entire liver (fig. 2), the cords of hepatocytes appear atrophic where as the phgocytic kupffer cells lining

the sinusoids are swollen, the nuclei of which appear as very small. There is abundance of young fibrous tissue in the picture (some fixation shrinkage has occurred), parts of sections show fibro-connective tissue bands are seen, those are thin septa with scantly in flammation cells in filtration, complete absence of hepatocellular structure lie not any lobule of liver (fig. 3) was differentiated or liver structure obscure lie fibrosis, no any which that indicate to a trial area of liver, loose capsulated areas of hydropic degeneration.

The spleen crossly is greatly enlarged firm. Microscopic lesion; the red pulp is largely occupied by collection of macrophages (histocytes). There are comparatively few sinusoids but large numbers of follicular structures. There is interfollicular tissue a hyalinized blood vessel with a thick hyaline wall. Multiple areas of diffuse lymphocytes arranged in tight concentric circles. The follicular tissue consist of an extensive network of small Bl. V. with eosinophilic hyalinized wall and small lumen (fig. 4), between them many small lymphocytes; some of them forming a clusters. Highly distention of the sinusoids. White pulp itself was loosed and disorganized; multiple areas of necrosis and found destroying the normal architecture of the white pulp (fig. 5). Proliferation of the sinus endothelial cells. In the Cytoplasm of some histiocytes, hemosidrin pigments were found (fig. 6). Also the fig. appears necrotic cells, reveals marked fibrosis in the Malpighian corpuscle.

(Fig, 1, 2, 3 in liver; Fig. 4, 5, 6 in spleen)

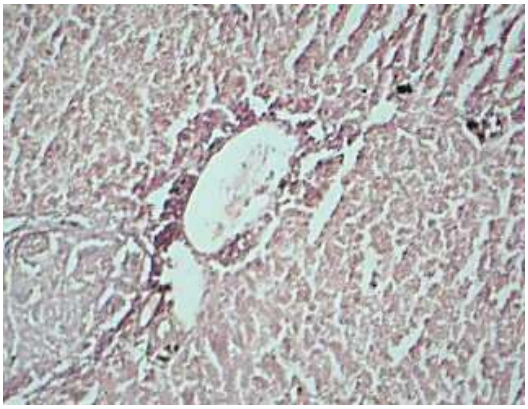

Fig. 1

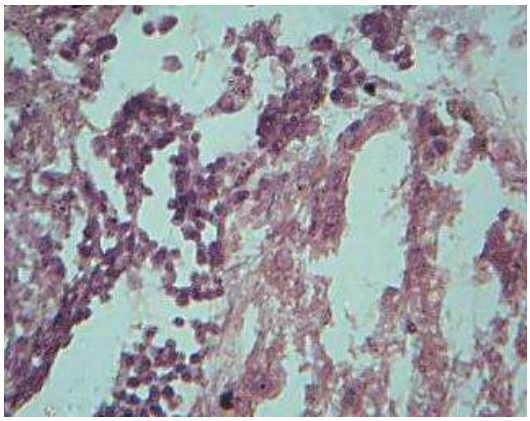

Fig. 2

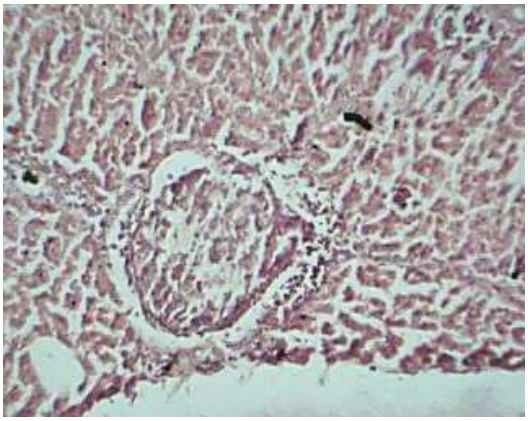

Fig. 3

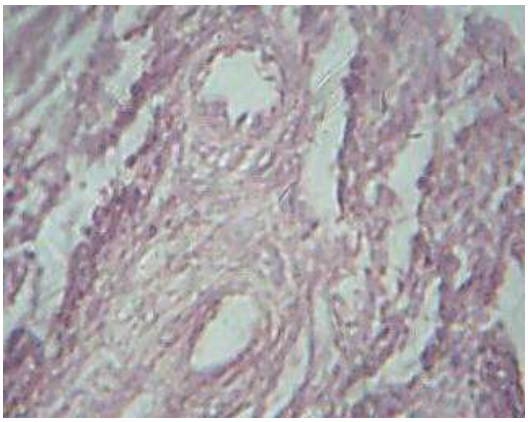

Fig. 4

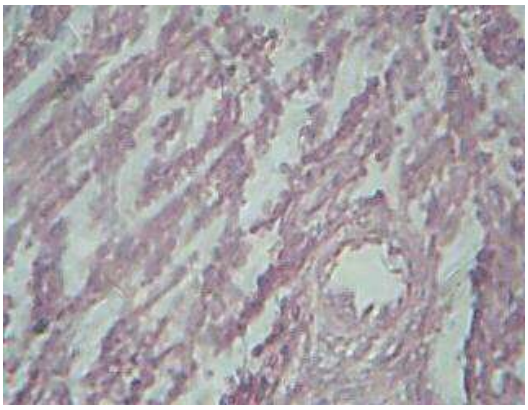

Fig. 5

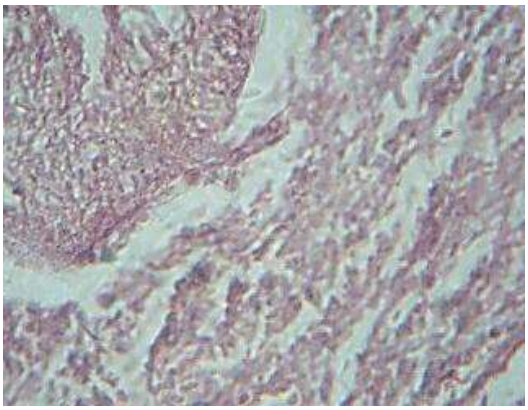

Fig. 6

Fig. 1: The figure shown part of a pseudo-lobule without a central vein there is peripheral portal trial with minute fatty change, the sinusoids are clearly dilated and congested. There is parenchymal inflammatory cells infiltration mostly lymphocyte.

Fig. 2: the figure shows connective tissue bands and septa with little of inflammatory cell infiltration mostly lymphocytes and plasma cells. The normal lobular architecture of the liver, parenchyma is absent, few necrosed hepatocytes, blood vessels are thickened.

Fig. 3: the figure shows middle layer these are no hepatic cord was appear like arrangement of the hepatocytes, the liver parenchyma is absent instead bizarre hepatocytes are seen, also picture showing bile pigment within or inspissated between them.

Fig.4: The figure shown inter-follicular tissues consist of an extensive net work of blood vessels with eosinophiles hyalinized wall of small lumen.

Fig. 5: the figure shows highly distention of the splenic sinusoids. White pulp it self was loosened and disorganized, multiple areas or necrosis found destroy the normal architecture of white pulp.

Fig. 6: the figure shows plasma cells infiltreation, proliferation of the sinus-endothelial cells, in cytoplasm of some histocytes, hemosidrin pigment were found.

Discussion

Canine visceral leishmaniasis is chronic disease of great epidemiological importance, the dog being the reservoir for human disease. Changes in liver and spleen associated with VL have been previously reported in various studies. However, the current study presents a systematic view of changes in different compartments of the liver and spleen in naturally infected dogs from an endemic area of VL.

Moreover, there was a positive correlation between hepatic fibrosis areas in naturally infected dogs and the parasite burden, appeared in our study, observed in other studies. These fibrous areas shown various thicknesses, being thicker in certain lobular areas than in others. In contrast, a peculiar and diffuse intralobular compatible with description by[12].

The presence uniform sized pseudolobules, scantly inflammatory cells infiltration, and complete absence of hepatocellular structure owing to fibrosis in numerous section mostly intralobular deposition were observed in portion of them by other workers [12, 13].

Perisplenitis is a common finding in canine VL [14,15]. Although the increase in spleen size may predispose that organ to trauma or mechanical stress, the actual genesis of perisplenitis in leishmaniasis remains unknown. It is interesting, however, that the perisplenitis was associated with the presence of parasite-containing macrophages in subcapsular areas. The presence of such macrophages in capsular and subcapsular inflammatory infiltrates has also been observed in

other studies [15]. These findings may indicate a role for inflammation in parasite dissemination [16,117], or that the parasite elicits an inflammatory reaction.

In light of previously published data regarding immunity to *Leishmania*, it seems contradictory that splenic granulomas were more frequently found in the group of infected animals with negative LST than in infected animals with positive LST. In effect both, granuloma and positive LST may result from DTH reactions to *Leishmania* antigens. The granulomas observed in the spleen of the animals in this study varied from poorly structured aggregates, consisting of at least four large epithelioid macrophages, to the barely organized structure shown in.The increase of macrophages in red pulp that observed may be determinant for immune response of host to VL. Hence, as observed in humans with long duration VL [18], these granulomas appear to reflect a persistent chronic inflammation in response to uncontrolled infection.

In fact, interlukin-10 and other cytokines produced by granuloma cells may provide conditions for the survival of *Leishmania* [19] and other microorganisms [20]. The fact that granulomas were only present in animals with positive spleen culture in our study supports the view that these granulomas may reflect a state of inefficient control of *Leishmania* infection by the host immune system.

References

1- World Health Organization. (1990): Control of leishmaniasis. *Tech. Rep. Series*; 793.

2- Keenan, CM. Hendricks, L.D, Lightner, L.; Webster, HK. And Johnson, AJ; (1984): Visceral leishmaniasis in the German shepherd dog. I. Infection, clinical disease, and clinical pathology. *Vet. Pathol*; 21: 74-79.

3- Badaro R; Jones, T.C.; Lorenco, R.; Cerf, BJ.; Sampaio, D.; Carvalho, EM.; Rocha, H.; Teixeira, R. and Johnson, WD. (1986): A prospective study of visceral leishmaniasis in an endemic area of Brazil. *J. Infect Dis.*;154(4):639–649.

4- Grech, V.; Mizzi, J.;Mamgion, M.and Vella, C. (2000): visceral leishmaniasis in Malta an 18 year paediatric, population based study. *Arch. Dis. Child*; 82:381-385.

5- Caldas, AJ.; Silvia, DR.; Pereira, CC.; Nunes, PM.; Silva, BP.; Silva, AA.; Barral, A. and Costa, JM.(2001): [*Leishmania* (*Leishmania*) *chagasi* infection in children from an endemic area of visceral leishmaniasis in the Sao Luis Island-MA, Brazil] *Rev. Soc. Bras. Med. Trop.*;34(5):445–451.

6- Pozio, E.; Gradoni, L.; Bettini, S.; and Gramiccia, M.(1981):. Leishmaniasis in Tuscany (Italy). VI. Canine leishmaniasis in the focus of Monte Argentario (Grosseto) *Acta Trop.*;38(4):383–393.

7- Seaman, J, Mercer, AL. and Sondorp, E. (1996): The epidemic of visceral leishmaniasis in western Upper Nile, southern Sudan: course and impact from. 1984 to 1994. *Int. J. Epidemiol.*;25(4):862–871.

8- Veress, B.; Omer, A.; Satir, AA. and El Hassan, AM. (1977): Morphology of the spleen and lymph nodes in fatal visceral leishmaniasis. *Immunology.*;33(5):605–610.

9- Ciaramella, P.; Oliva, G.; Luna, RD.; Gradoni, L. Ambrosio, R.; Cortese, L.; Scalone, A. and Persechino, A. (1997): A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. *Vet. Rec.*; 141(21):539–543.

10- Barrouin-Melo, SM.; Larangeira, DF.; de Andrade, FA.; Trigo, J.; Julião, FS.; Franke. CR.; Palis Aguiar, PH.; Conrado, dos-Santos, WL. aand Pontes-de-Carvalho. L.(2006): Can spleen aspirations be safely used for the parasitological diagnosis of canine visceral leishmaniosis? A study on asymptomatic and polysymptomatic animals. *Vet. J.*; 171(2):331–339.

11- Carrion, J. Nieto, A. and Iborra, S.; Iniesta, V.; S;oto, M.; Folgueira. C.; Abanades, DR,; Requena, JM. and Alonso, C. (2006): Immunohistological features of visceral leishmaniasis in BALB/c mice. *Parasite Immunol*.; 28(5):173–183.

12- Corbett, C.E.; Duarte, M.I. and Bustamaute, SE. (1993): Regression of diffuse intralobular liver fibrosis associated with visceral leishmaniasis. *Am. J. Trop. Med. Hyg*., 49:616-624.

13- Ferdinan, M.; Marina, M.; Patricia, O.; Wanderson, L.; Mariana, A.; Marilene, M.; Pedero, R.; Washington, T. and Wagner, T. (2008): Diffuse intralobular liver fibrosis in dogs naturally infected with leishmania. *Am. J. Med. Hyg*., 79(2): 198-204.

14- Tafuri. WL.; Barbosa, Aj.; Michalick, MS., Genaro, O. Franca, IC.; Mayrink, W. and Nascimento, E. (1996): Histopathology and immunocytochemical study of type 3 and type 4 complement receptors in the liver and spleen of dogs naturally and experimentally infected with *Leishmania* (*Leishmania*) *chagasi*. *Rev. Inst. Med. Trop. Sao. Paulo.*; 38(2): 81–89.

15- Tryphonas, L.; Zawidzka, Z.; Bernard, MA. and Janzen, EA. (1977): Visceral leishmaniasis in a dog: clinical, hematological and pathological observations. *Can. J. Comp. Med.*; 41(1):1–12.

16- Bertho AL.; Santiago, MA.; and Coutioho, SG. (1994): An experimental model of the production of metastases in murine cutaneous leishmaniasis. *J. Parasitol*.; 80(1):93–99.

17- Dos-Santos, WL.; David, J.; Badaro, R. and de-Freitas, LA. (2004): Association between skin parasitism and a granulomatous inflammatory pattern in canine visceral leishmaniosis. *Parasitol Res.*; 92(2):89–94.

18- Daneshbodm K. (1972): Visceral leishmaniasis (kala-azar) in Iran: a pathologic and electron microscopic study. *Am J Clin Pathol*.; 57(2):156–166.

19- Wilson, ME.; Sandor, M.; Blum, AM.; Young, BM.; Metwali, A.; Elliott, D.; Lynch RG. And Weinstock, JV. (1996): Local suppression of IFN-γ in hepatic granulomas correlates with tissue-specific replication of *Leishmania chagasi*. *J. Immunol*.; 156(6):2231–2239.

20- Fenhalls, G.; Stevens, L.; Bezuidenhout, J.; Amphlett, GE.; Duncan, K.; Bardin, P. and Lukey, PT. (2002): Distribution of IFN-γ, IL-4 and TNF-α protein and CD8 T cells producing IL-12p40 mRNA in human lung tuberculous granulomas. *Immunology*.; 105(3): 325–335.