ISSN 1991- 8690 الترقيم الدولي ٨٦٩٠ - ١٩٩١
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Experimental study of the pattern infection with cutaneous leishmaniasis between males and females of mice

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Abstract

In the present study the development of skin lesions in males and females BALB/c mice infected with *Leishmania major* strain was investigated. The results of this study demonstrated that the infection with cutaneous leishmaniasis begins in females before males and there are differences in appearance and development of skin ulcers was found between males and females of mice throughout the experiment (6 months). The heavily infected parts were completely lost from the mice body. The metastatic cutaneous lesions were observed on both sex males and females.

Introduction

Cutaneous leishmaniasis (CL) was an endemic disease in Iraq. It is mostly seen in Baghdad (pringle, 1957), it is known as " little sister" in countries where the disease is so common that it is part of the family (WHO, 1998). (CL) is caused by two species of *Leishmania, L.major* causing a rural wet type with early ulceration and the other is *L.tropica* causing an urban dry type that runs a chronic course with late ulceration (Sukker, 1983). The disease starts with a papule that enlarged and become ulcer.

The lesions may be single or multiple, diffuse lesions may heal spontaneously within weeks to months or last for a year or more (Chin, 2000 ; WHO, 2003). Ulceration may leave large disfiguration scars (Garcia & Bruckner, 1993).The pathogenicity of the above organisms also differs in various laboratory animals *L.major* infects mice and golaen hamsters whereas species of *L.tropica* are only infective for golden hamsters but not for mice (Nadim *et.al*., 1968). The aim of the present work is to study the pattern of infection with CL and development of metastatic disease caused by *L.major* strain between males and females mice.

Materials and Methods

The strain of CL which used in the present study was clinically identified as *L.majo*. This strain was isolated from male patient aged 23 years with a disease history 3 months by specialized dermatologist. Aspirate material from ulcer marginwas spread on a pre-cleaned slide. The prepared smear was stained

with leishman's stain. Amastigote was seen under the light microscope.The promastigotes were cultivated in Nicolle-Novy-MacNeal (NNN) medium (Kagan and Norman, 1970; Meredith *et. al*., 1995) at (26-28°C) then harvested on the 6th day for animals infection. BALB/c mice (8-10) weeks oldwith a body weight of approximately 20-25 gm were used in this study. The promastigotes washed three times with saline counting with hemocytometer and then adjusted to $1x10^7$/ 0.1 ml for inoculation (Hazra *et. al.*, 1987).

Fourty BALB/c mice were inoculated with $1x10^7$/ 0.1 ml of promastigotesin a shaved area above the tail and at the hind footpad (each footpad received (0.05 ml). Ten clean cages was prepared, each cage contain 4 mice (2 male and 2 female). The infected animals were monitored periodically for morphological signs of CL such as erythema, papula, swelling and redness. Aspirate material was taken from the margin of ulcer smeared on a clean slide and stained with leishman's stain, then examined under oil immersion to detect the presence of amastigotes. Material aspirate from cutaneous lesions after local injection with locks solution were cultured under aseptic condition on diphasic (NNN) medium, care was taken from any contamination, the development of promastigote was checked microscpically for 2 weeks. The time for appearance of infection, the development of large pathogenicity and metastatic cutaneous lesions were monitored.

Result

The total mice infected with *Leishmania major* in this study were 40 (20 males and 20 females) after 6 months post infection (Table 1). All mice were infected with CL except 3 male at the end of the experiment. The males mice have small sized lesions, while females showed bigger sized ulcer. Eleven females showed infected with CL after 4 weeks post infection. Some mice were lost some parts of their bodies due to heavy infected with *L.major* (Table 2) and Figure (1). The picture of the mice with metastatic cutaneous lesions on back and face Figure (2). The cutaneous metastatic lesions were observed on both sex males and females and detected at late stage of infection or acute stage (Table 3).

Table (1): Clinical manifestation of mice infected with cutaneous leishmaniasis (40 mice, 20 males and 20 females)

Duration of post infection	Sex	No. of infected mice
1 months	Male	0
	Female	11
2 months	Male	5
	Female	1
3 months	Male	3
	Female	3
4 months	Male	1
	Female	4
5 months	Male	-
	Female	3
6 months	Male	-
	Female	6

Table (2): Cutting parts of mice infected with cutaneous leishmaniasis

No. of cases	Sex	The part lost
3	Female	Tail from base
2	Male	Tail from base
2	Female	Right foot
1	Female	Left foot
1	Male	Left foot
1	Female	Tip of tail

Table (3): Relationship among metastatic disease, sex and place of lesion

No. of cases of metastatic disease	Sex	The place of the lesion
1	Female	On the back
5	3 females + 2 males	Diffuse to tail region
2	Female	On the face

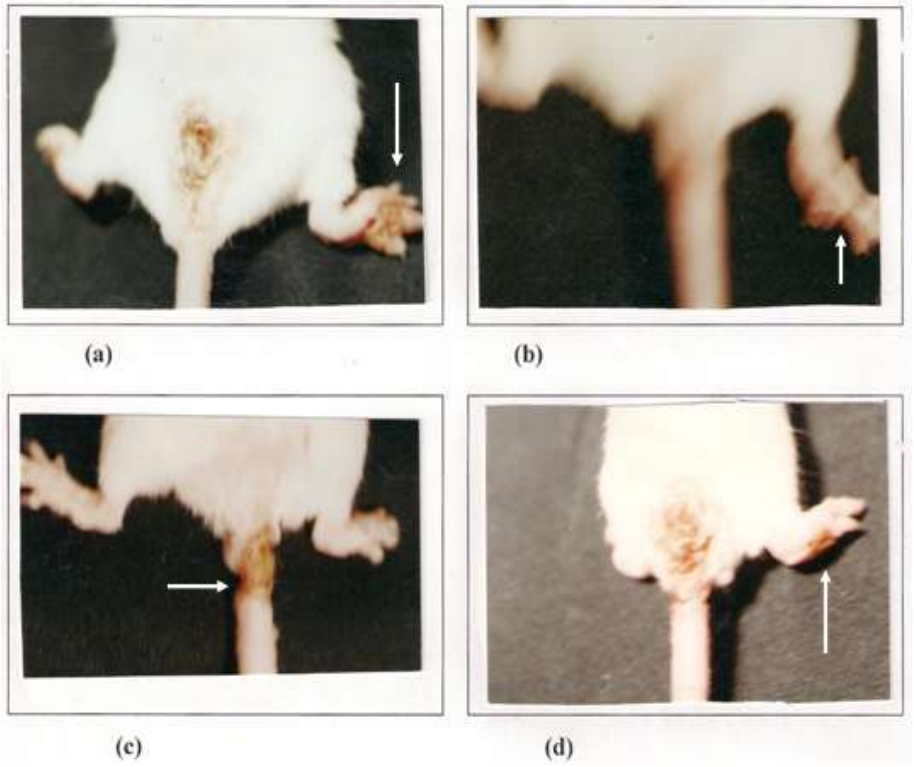

Figure (1): Heaving infected mice with *Leishmania major*
a) Right foot b) Left foot c) Tail d) Left foot

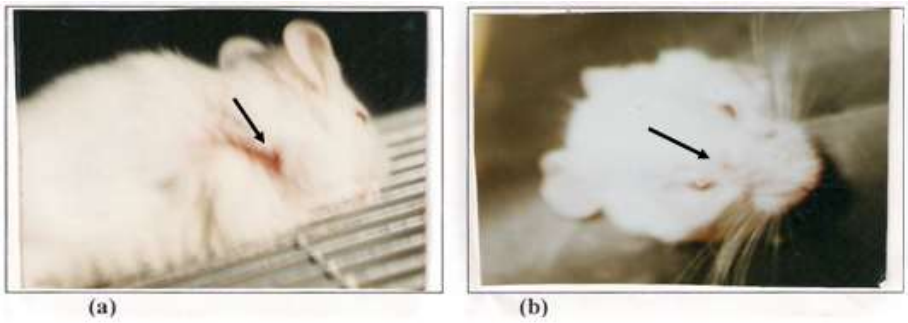

Figure (2): Shows two mice with metastatic cutaneous lesion
a) On back b) On face

It was found from the present study that the pattern of infection and the pathogenicity of *L.major* is quite different between males and females of BALB/c mice even each sex has received the same dose of promastigote stage. The result present here demonstrated that the infection with CL begins in females before males. Even females mice show the appearance of infection with *L.major* after 4 weeks post infection, while only one male shows the appearance of infection after 8 weeks post infection. Skin lesions were noticed at short time on females mice compared with males. The males have shown small sized lesions while females mice showed bigger sized ulcer. The metastatic disease of distant cutaneous site was noticed on these mice during the present study. Salit *et. al.* (1998) showed that the lesion started as a small nodule that soon ulcerated and began to spread overs the caudual part of the back and extended posteriorly over the perianal skin and often ventrally up to the urethra.

Cutaneous leishmaniasis in Basrah may differ from other areas. This differences may be related to the species, vectors, environments and ecological factors, this differences may be related to the biochemical, biological characteristics of sex of mice or it may be related to the decrease of the immunity system in females during the pregnancy period which lead to occurrence early infection with disease. It was demonstrated that CL caused by *L.major* transmission from infected mated BALB/c mice to their babies through the intra-uterine placentation (Awad & Jarallah, 2006). Matte & Olivier (2002) reported that *L.major* showed strong induce of the early inflammatory response compared with *L. donovani.*

The lost parts from some heavily infected animals (at site of inoculation) may be due to the development of the pathogenicity of the disease in these sites or it may be related to the secondary bacterial and fungal infections. It was found that cutaneous leishmaniasis is caused by two different species of *L.major* and *L. tropica* (Moaddeb *et. al.*, 1993). In desert storm, the infection with *L. tropica*. Parasites caused viscerotropic and cutaneous disease in soldiers (Kreutzer *et. al.*, 1993), that is mean both parasite and patient can play important roles in disease manifestation.

Youssef *et. al.* (1996) reported that cutaneous metastatic lesions development exclusively on the tail and the face of mice experimentally infected with *L. tropica*. In recent study proved

that the *L.major* disseminate from site of inoculation to distant cutaneous sites and visceral organs such as liver, spleen, and kidney (Jarallah, 2003). It was reported that the disseminate of *L. tropica* in distant cutaneous sites found only in two albino mice and at the 16th weeks post infection. The immunity of parasite and the physiological properties influence in a major way the pattern of development of disease (Youssef *et. al.*, 1996).

References

Awad, A.H. and Jarallah, H.M. (2006). The intra-uterine transmission of *Leishmania major* in BALB/c mice. J. Bas. Res. 32:32-34.

Chin, J. (2000). Control of communicable disease manual 17th ed. An Official report, American Public Health Association. Pp580.

Garcia, L.S. and Bruckner, D.A. (1993). Diagnostic Medical Parasitology. American Society for Microbiology. Washington D.C. Pp. 764.

Hazra, B.; Saha, A.K.; Ray, R. *et al*. (1987). Antiprotozoal activity of diospyrin towards *Leishmania donovani* promastigotes *in vitro*. Roy. Soc. Trop. Hyg., 81:738-741.

Jarallah, H.M. (2003). Effect of some plant extract and antibiotics with histopathological study on *Leishmania major* strain. M.Sc. Thesis. Edu. Coll. Univ. Basrah. Pp116.

Kagan, I.G. and Norman, L. (1970). Manual of clinical microbiology. Am.Soc. Microbiol., Washington, USA. Pp. 479.

Kreutzer, R. D. ; Grogl, M. ; Neva, F. A. *et. al.*, (1993). Identification and genetic comparison of leishmanial parasites causing viscrotropic and cutaneous disease in soldiers returning from operation desert storm. Am. J. Trop. Med. Hyg., 49(3): 357-363.

Matte, C. and Olivier, M. (2002). *Leishmania* induced cellular recruitment during the early inflammatory response: modulation of proinflammatory mediators. Infec. Dis. Soc. Am., 185: 673-681.

Meredith, S.E.; Kroon, C.M.; Sondorp, *et al*. (1995). Leish-KIT, astable DAT based on freeze dried antigen for serodiagnosis of visceral leishmaniasis. J. Clin. Microbiol., 33:1742-1745.

Moaddeb, A; Gettner, S. AND Ardehali, S. (1993). Studies on thecausative agent of cutaneous leishmaniasis in Shiraz- Iran. Iran. J. Med. Sci. 18: 28-33.

Nadim, A.; Seyedi-Rashti, M.A.; Mesghali, A. (1968). Epidemiology of cutaneous leishmaniasis in Turkemansahra.Iran.J.Top.Med.yg. 71: 238-239.

Pringle, G. (1957). Oriental sore in Iraq: Historical and epidemiological problems. Bull. End. Dis., 2: 41-76.

Salti, N.N. ; Matta, M.; Shbaklo, Z.; Lakkis, M.and Kabbani, Z.E. (1998) Behavior in a mouse model of isolated of *Leishmania donovani sensu lato* cultured from the blood of patients with cutaneous leishmaniasis. Am. J.Trop. Med. Hyg.58: 710-714.

Sukker F. (1983). Epidemiology of leishmanisis in Iraq. Bull. End. Dis., 22, 33:50.

World Health Organization. (1998). *Leishmania* and HIV. WHO/CTD/Leish, UNAIDS. 23.

World Health Organization. (2003). WHO communicable disease profile for Iraq. 39-43.

Youssef, M.Y.M.; Eissa, M.M.; el-Naga, I. F.A. *et al*., (1996). Dissemination of *Leishmania* to organs of mice experimentally infected with *Leishmania tropica*. J. Egypt. Soc. Parasitol., 26: 719-731.

دراسة تجريبية في تطور الافات الجلدية في اناث وذكور الفئران المصابة بداء اللشمانية

هند مهدي جار الله

مركز علوم البحار- جامعة البصرة

الخلاصة

تضمنت الدراسة الحالية البحث في تطور الآفات الجلدية في إناث وذكور الفئران ألمختبريه نوع BALB/c المصابة بسلالة *Leishmania major* . برهنت نتائج هذه الدراسة على إن الاصابه بداء اللشمانيا الجلدية بدأت في الإناث قبل الذكور ووجد هناك اختلاف في ظهور وتطور القرح الجلدية بين إناث وذكور الفئران خلال التجربة (٦ أشهر). الأجزاء المصابة الخطيرة فقدت تماما من جسم الفئران. لوحظت الافات الجلديه النقيله على كلا الجنسين في الاناث والذكور.