The pathogenicity of *Trichomonas vaginalis* in experimentally infected Balb/c mice

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Abstract

In the present study a Balb/c mice were investigated for its susceptibility to infection with cultivated strain of *Trichomonas vaginalis*, which recovered from infected women. Animals were inoculated through subcutaneous route.

The current results indicated that the strain was virulent by producing liver abscess as confirmed by histopathological study, which revealed the presence of the parasites within the damaged liver cells beside infiltration of different inflammatory cells like eosinophils, macrophages, polymorphonuclear cells and lymphocytes. Moreover hemorrhage was also detected in the necrotized area.

الامراضية للطفيلي *Trichomonas vaginalis* في فئران Balb/C المصابة مختبرياً

بشرى حسين شناوة ، وفاء سعدون شاني و مها خليل الملاك
قسم علوم الحياة ، كلية العلوم ، جامعة البصرة

الخلاصة

في الدراسة الحالية لوحظ بان فئران Balb/C لها القابلية على الاصابة بالسلالة المستزرعة من طفيلي ***Trichomonas vaginalis*** والماخوذ من النساء المصابات بالطفيلي.

اثبتت النتائج الحالية بان هذه السلالة من الطفيلي تمتلك ضراوة لها القدرة على احداث خـراج وكما هو مبين في الدراسة النسيجية الامراضية التي اوضحي وجود الطفيليات ضمن خلايا الكبد المحطمة اضافة لترشيح مختلف الخلايا الالتهابية مثل الحمضيات وخلايا متعددة الاشكال النووية والخلايا اللمفاوية . اضافة لذلك فقد تم ملاحظة وجود النزف في المنطقة المتنخرة.

Introduction

Trichomonas vaginalis is a protozoan parasite that infects the human urogenital tract and is the causative agent of trichomoniasis (Petrin, etal., 1998). This disease is a problematic sexually transmitted mainly in women, where it may be asymptomatic or causes severe vaginitis and cervicitis (Snipes etal, 2000).There is some evidence that under unhygienic conditions , transmission may take place through soiled clothing or wash cloths , but such transmission is probably rare because trichomonad survive only a short time when exposed to the air (Marquardt et al., 2000) .

T. vaginalis infections have also been related to complications during pregnancy, including premature rupture of membranes (Minkoff, etal.1984), low birth weight and preterm labor (Cotch, etal.1997). Another serious aspect of this disease is the association between trichomoniasis and an increased risk of transmission of other sexually transmitted disease, including human immunodeficiency virus (Laga, etal.1993).

Schnitzer etal., (1950) studied the pathogenicity of *T. vaginalis* to mice and found that a single intra-peritoneal injection led to an infection after an incubation period of 8 days, and after 3weeks multiple small abscesses occurred in the peritoneal cavity .When this parasite was injected intra-muscularly it produced large abscesses in 2-3 days post-inoculation. Also subcutaneous injection produced abscesses in the treated mice .

Furthermore, Caterina etal., (1996) confirmed a fact ,that a biological assay based upon the induction of abscesses in mice injected subcutaneously with *T. vaginalis* was shown to be a valid method for comparing the virulence of isolates. Other worker proved that estrogenized mice which infected with *T. vaginalis* were a useful animal model for the study of virulence factors (Corbeil, 1995).

In light of the high virulence of *T. vaginalis* in Iraq beside the little or the lack of enough information about the animal model of trichomoniasis here, which may be useful for the study of virulence factors or chemotherapy resistant. Therefor, an attempt was made to culture and

studied the ability of the parasite to induce any histopathological effects in subcutaneously injected Balb/c mice after three weeks post injection.

Materials and Methods

Trichomonas vaginalis isolate was recovered from vaginal swab obtained from women suffering from severe vaginitis attending special clinics in Basrah city. The parasite was identified in the vaginal exudate by direct microscopical examination of a wet smear.

Cultivation of the parasite

Specimens of the parasite were inoculated into filter sterilized broth medium (Feinberg & Whitting ton, 1957) contained liver powder 2.5g sodium chloride 0.65g, Glucose 0.5g, penicillin G 1000000 units, streptomycin 500000 units, which dissolved in 100 ml distilled water and the PH was adjusted to 6.4 . Then aseptically 8% of heat - inactivated (56C° for 30min) bovine serum was added, and stored in screw-capped tubes at 37°C. The culture tubes were examined daily and subcultured every three-day by centrifugation of the culture and the addition of the sediment to a new culture media.

Inoculation of Balb/c mice

Six female mice were injected subcutaneously with 0.5 ml of sediment from centrifuged culture. The animals were sacrificed after three weeks post inoculation, and examined directly for the infection. Pieces of liver tissue lesions were fixed in 10% formalin buffer and embedded in paraffin wax. Four µm thick, tissue section were stained with haematoxylin and eosin techniques (Luna, 1968) then examined and compared with section of control animals which inoculated with normal saline.

Results

The results of the present study indicated that the trophozoites of *T. vaginalis* were established successfully in culture by explanting aliquots of vaginal exudate containing the parasite into fresh prepared medium

supplemented with antibiotics. The parasite was motile and intact as in direct microscopic examination figure (1).

Moreover, the present data also determined the ability of *T. vaginalis*, which isolated from symptomatic cases to produce severe infection in mice when compared with control group.

The results of histopathological study indicated a formation of gross liver ulceration and the microscopic examination of the lesion showed the presence of pus cells, trophozoites and necrotic material. Also the histopathological section showed the presence of the parasite in the liver tissue within the damaged hepatocyte which seemed to be necrotized with densely staining nuclei that known as pyknosis figure (2,3).

Furthermore, the tissue section also revealed the infiltration of different inflammatory cells like eosinophils, macrophages, lymphocytes, polymorphonuclear cells. Figure (4, 5, 6) in addition to occurrence of hemorrhage. Figure (7), in comparison to the control mice, which did not show any histopathological changes.

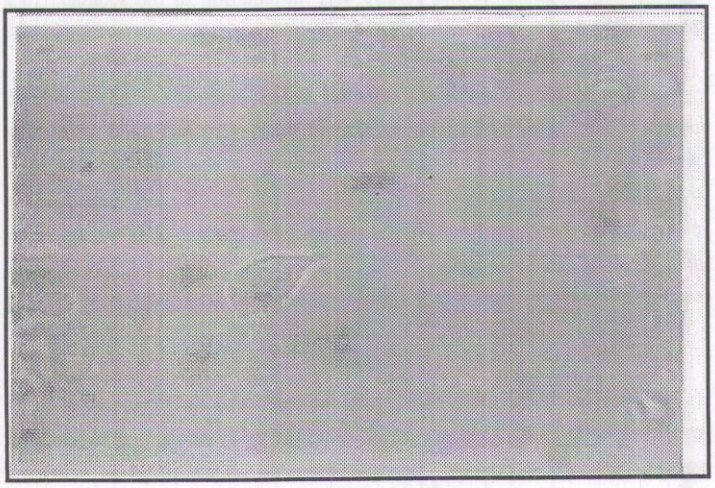

Fig. (1) Trophozoite of *T. vaginalis* in culture media. (X1000)

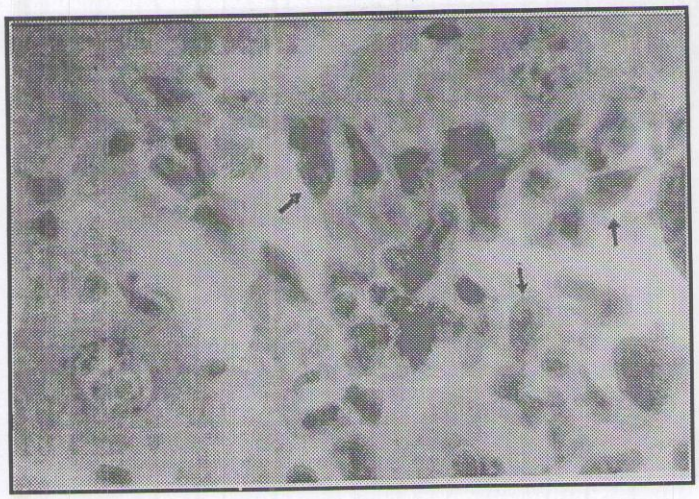

Fig. (2): Trophozoite of *T. vaginalis* in necrotized hepatic tissue. (⟶).

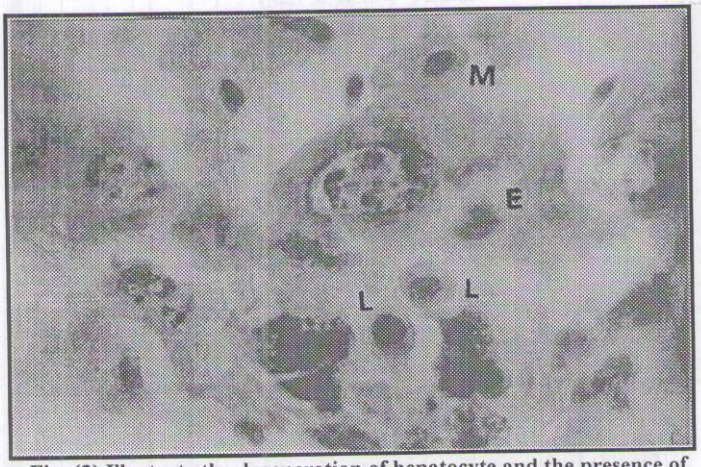

Fig. (3) Illustrate the degeneration of hepatocyte and the presence of eosinophils E, Lymphocytes L, Macrophage M (X 1450) .

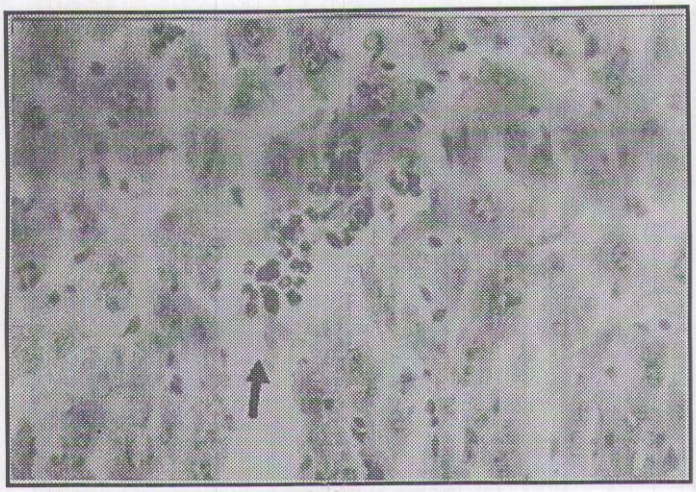

Fig. (4) Intense inflammatory inflammation in liver of infected mice. (⟶) (X 725)

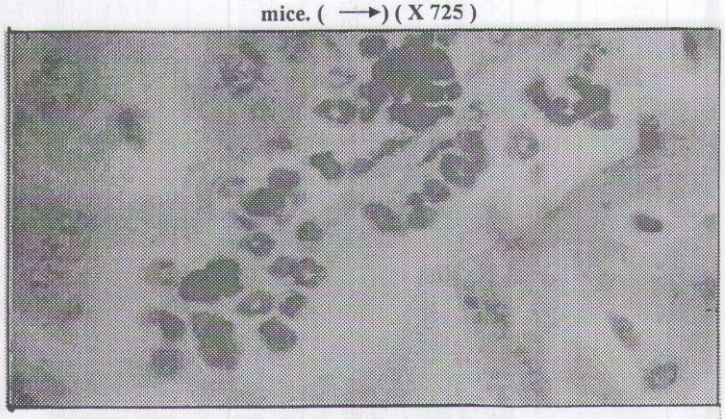

Fig. (5) High magnification of the fig. (4) showed the heavy inflammation. (⟶) (x 1220)

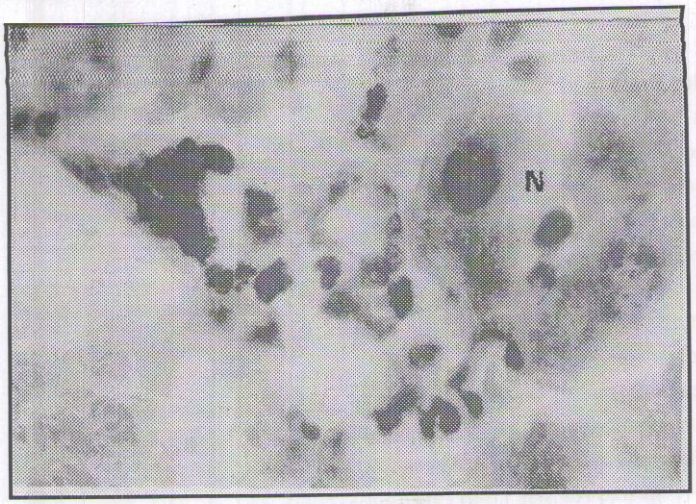

Fig. (6) Necrotized hepatocyte with the presence of inflammatory cells. (N) (x 1350)

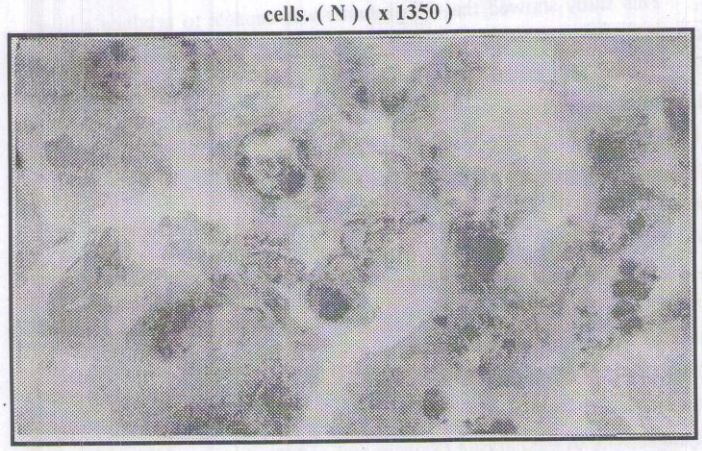

Fig. (7) High magnification picture showed the hemorrhage with liver cells of infected mice. (x 1360)

Discussion

The present study reported the ability of the diagnosis and identification of *T. vaginalis* from vaginal discharge by wet smear method, as well as by culturing on Feinberg-Whittington medium. This medium showed to be suitable for cultivation the parasite. Robertson etal., (1969) concluded that cultivation of *T. vaginalis* is a more sensitive diagnostic method than either immediate microscopy of a wet mount or of the centrifuged deposit.

Moreover, this study succeeded in the establishment of an axenic culture of *T. vaginalis* by the addition of antibiotics and nystatin to the medium in order to remove the bacteria and the fungus *Candida* respectively, which were detected in the direct microscopic examination of the vaginal exudate. Also, subculturing seemed to be very important to minimize the above-mentioned microorganism from the parasite medium.

In Iraq Almalah (1981) also succeeded in culturing the same parasite by using (Trichomonas medium No. 2).

This study showed that *T. vaginalis* was capable to produce a liver abscesses in subcutaneously injected mice and this may be related to the high virulence of parasite isolates. Besides its cultivation in liver digest medium that containing iron. This fact was proved previously by (Ryu etal., 2001) whom studied the effect of iron on the virulence of *T. vaginalis* in subcutaneously injected mice. The same workers also showed that *T. vaginalis* grown in an iron - deficient medium failed to produce any pathology.

Early reports indicated a generally good correlation between clinical symptoms and pathogenicity as demonstrated by the subcutaneous mouse assay (Honigberg, 1961). And there is evidence that the pathogenicity of trichomonads may be correlated with surface antigens (Su-Lin and Honigberg, 1983), surface saccharides (Warton and Honigberg, 1983) and hemolytic activity of the parasite (Krieger etal., 1983).

Furthermore, the current study proved the presence of numerous *T. vaginalis* trophozoite in liver tissue, which surrounded by heavy

inflammatory infiltration, especially eosinophils, polymorphonuclear leukocyte and lymphocytes.

It was evident that leukocytes play an important role in trichomonad pathogenicity. Their breakdown not only appears to be related to the lysis of host tissues, but also may actually provide nourishment for the parasites (Frost and Honigberg, 1962).

Also Reardon etal., (1961) described the liver lesions of mice injected intraperitoneally with Tvc1 strain of *T. vaginalis*. According to these workers, there was always a band of parasites at the necrotic lesion as it progressed through the liver. Moreover, there were infiltrating inflammatory cells principally polymorphonuclear leukocytes. In the same point, Roitt etal., (1998) concluded that neutrophils play an important role in the immune response against large and small parasites. These cells are phagocytic and can produce a more intense respiratory burst than macrophages and their secretory granules contain highly cytotoxic proteins. Neutrophils are present in parasite-infected inflammatory lesions and probably act to clear parasites from bursting cells. It is worthwhile to mention that neutrophil is considered as characteristic cell in the early stage of acute inflammation. (Macsween and whaley, 1994). Furthermore, the result of Gani (2000) showed the presense of ascites and multiple abscesses on the visceral organs in mice injected intraperitoneally with alocal strain of *T. vaginalis* Also the results of the present study agreed with the finding of (Bhatt etal., 1997) whom proved the pathogenicity of seven isolates of *T. vaginalis* recovered from symptomatic cases in experimentaly infected mice .

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