

Infectious Complications of Visceral Leishmaniasis in Basrah, Southern Iraq

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Abstract

Objectives: A prospective study was carried out to study the complications that may be associated with visceral leishmaniasis with special emphasis on any bacterial or parasitic complications.

Methods: The study included 132 in-patient children who were admitted to Basrah Maternity and Children Hospital and Basrah General Hospital with visceral leishmaniasis from November 2004 till November 2005.

Direct agglutination test (DAT) and bone marrow examination were used to confirm the diagnosis. All patients were sent for blood culture, urine culture and stool culture, in addition to general stool examination.

Results: Among 132 sero-positive cases, Diarrhea with or without vomiting, was found in 75 cases (56.8%), followed by urinary tract infection in 30 (23.4%) of the cases, and bronchopneumonia in 10 cases (7.5%). The main non-infectious complications were malnutrition in 86 (65.1%) of the cases, followed by bleeding tendency in 18 cases (13.6%).

Pathogenic bacteria were isolated in 41(31%) out of 132 sero-positive patients. The commonest type of bacteria isolated was *Klebsiella* (16 isolates) followed by *Escherichia coli* and *Proteus* (11 and 9 isolates), respectively. These three types of bacteria represented (78.2%) of total isolates.

Parasitic infections were detected in 44 (33.3%) patients. The commonest parasites detected were *Cryptosporidium parvum* (18.9%) and *Entamoeba histolytica* (13.6%), *Giardia lamblia*, *Blastocystis hominis*, *Hymenolypis nana*, and *Enterobius vermicularis* were also detected but in lower frequency.

Conclusion: From this study we conclude that bacterial infections and pathogenic parasites were confirmed in about one third of VL cases for each; *Klebsiella* is the most common bacteria reported while *Cryptosporidium parvum* is the most common parasite.

ملخص

الاهداف: اجريت هذه الدراسة المستقبلية لمعرفة المضاعفات التي قد ترافق اللشمانيا الحشوية مع تشديد خاص على اي مضاعفات الجرثومية والطفيلية

الطريقة: ضمت الدراسة 132 طفلا تم ادخالهم إلى مستشفى البصرة للنسائية والأطفال ومستشفى البصرة العام للفترة من تشرين الثاني 2004 ولغاية تشرين الثاني 2005 .

تم التشخيص بواسطة اختبار التلازن المباشر وفحص نخاع العظم . تم اجراء زرع دم , زرع للادرار و البراز من جميع المرضى , اضافة الى تحليل البراز العام .

النتائج : من بين 132 مريضا اعطى تفاعلا مصليا موجبا, 75 (56.8%) مريضا كانوا مصابين باسهال مع او بدون تقيؤ, 30 (23.4%) من التهاب المجاري البولية, و 10 (7.5%) من التهاب رئوي . اهم المضاعفات غير الخمجية كانت سوء التغذية في 86 (65.1%) وقابلية النزف في 18 (13.6%) من المرضى .

تم عزل الجراثيم المرضية من 41 مريض (31%) من اصل 132 مريض وقد كانت جرثومة *Klebsiella*

هي الاكثر شيوعا (16 عزلة), تليها *Proteus* و *Escherichia coli* (11 و 9 عزلات). وقد مثلت هذه الانواع الثلاثة من الجراثيم (78.2%) من مجموع العزلات الكلي. تم تشخيص الاصابات الطفيلية المرضية في 44 مريض (23.2%). اكثر الطفيليات شيوعا كانت *Cryptosporidium parvum* (18.9%) ثم *Entamoeba histolytica* (13.6%).
عزلها ولكن بنسب اقل. *Giardia lamblia*, *Blastocystis hominis*, *Hymenolypis nana*,
Enterobius vermicularis و *Klebseilla* اكثر الجراثيم شيوعا, في حين كانت *Cryptosporidium parvum* اكثر الطفيليات شيوعا.
خاتمة: من هذه الدراسة نستنتج ان الالتهابات الجرثومية والطفيليات المرضية تم اثبات لاصابة بها في ثلث الاطفال المصابين باللشمانيا الحشوية.

Introduction

Leishmaniasis, a parasitic disease transmitted by the bite of some species of sandflies affects various age groups depending on the species, of *Leishmania* geographic location, disease reservoir, and host immunocompetence. Visceral leishmaniasis (VL) is the most severe form of the disease affecting children.⁽¹⁾

During the past few years, there were increases in visceral leishmaniasis cases in the southern parts of Iraq (Basrah, Maysan, Thi-Qar, Al-Qadysia and Al-Muthanna), it was reported that (72.7%) and (69.5%) of all recorded cases all over the country during the years 1995 and 1996, respectively, were from these governorates, (10.2%) of cases recorded were from Basrah.⁽²⁾

Visceral leishmaniasis patients present symptoms and signs of persistent systemic infection (including fever, fatigue, weakness, loss of appetite and weight loss) and parasitic invasion of the blood and reticulo-endothelial system (that is, the general phagocytic system), such as enlarged lymph nodes, spleen and liver.⁽³⁾

Cellular immune mechanisms determine resistance or susceptibility to infection with *Leishmania*. Resistance is mediated by expansion of the T helper 1 (Th1) cell population, with interferon- γ production resulting in macrophage activation and parasite killing. Susceptibility is associated with expansion of Interleukin 4(IL-4) producing T-helper 2(Th2) cells and /or the production of (IL-10) and transforming growth factor- β (TGF- β)

which is potent inhibitors of macrophages activation.⁽³⁻⁵⁾

The crucial role of the cell mediated immune response is illustrated by the increased risk of developing clinical illness in cases of malnutrition or concomitant immunosuppressive diseases, such as HIV infection. Other risk factors for developing clinical illness have been identified and include young age, decreased production of interferon-gamma and polymorphisms in the promoter of the tumor necrosis factor-alpha gene.⁽³⁾

Bacterial infections (pneumonia, septicaemia, otitis media, urinary tract infections and skin infections) are common complications. Bacterial superinfection is one of the major complications leading to death in children with VL. Parasitic infestations of the gut are very common in paediatric VL patients.⁽⁶⁾

The major complications leading to death, including hemorrhage and bacterial superinfection, result from a decrease in blood elements due to leishmaniasis of the bone marrow and hypersplenism.⁽⁷⁾

This study was carried out to study the complications associated with visceral leishmaniasis with special emphasis on bacterial and parasitic complications among these patients.

Patients & Methods

Patients:

This study is a prospective study that was carried out over 16 months (from the first of November 2004 till the end of October 2005), on children with visceral

leishmaniasis who were admitted to pediatric wards of Basrah Maternity and Children Hospital and Basrah General Hospital.

The diagnosis was confirmed by Direct Agglutination Test (DAT), in addition bone marrow examination was done for them. A total of 132 patients were included.

Data Collection:-

The following clinical data were recorded from each patient; age, sex, residence, history of fever, pallor, jaundice, cough, vomiting, abdominal distention, loss of appetite, loss of body weight and diarrhea. Physical signs included confirmation of fever, pallor, jaundice, hepatosplenomegaly, and lymphadenopathy.

Weight for length was assessed using the National Center for Health Statistics (NCHS)/WHO normalized reference values charts. (8)

An informed consent from one of the parents was obtained before enrollment in the study. The study was approved by the Ethical Committee and College Council of Basrah Medical College.

These patients were followed and the complications were recorded. The final diagnosis and the outcome of the patients with VL were recorded also.

Sampling and Examination:

Blood Samples:

Two to three ml (2-3 ml) of blood was collected, and cultured in enrichment media (Brain Heart infusion broth) for 24 hrs and in the second day was sub-cultured on blood agars and MacConkey media⁽⁹⁾, to study the bacterial complications that may accompany VL.

Another two ml of blood which was collected is allowed to clot at room temperature then centrifuged at 3000 r.p.m. for 5 min. Serum was stored in deep freeze at -20°C until used in (DAT).⁽⁹⁾

Urine Samples:

Urine samples were collected from each patient. Each sample was cultured only aerobically on MacConkey medium and blood agar and incubated at 37°C for

24hrs; in the second day the culture was examined, biochemical reactions and gram stain, were done also^(9,10) to determine the bacterial complications that may accompany VL.

Fresh urine samples were also centrifuged at 1800 r.p.m. for 3 min., the supernatant fluid was poured off and a drop from the deposit was taken on a slide and covered with cover slip and examined under high power objective lenses⁽⁹⁾ to look for RBC, pus cells, epithelial cells and other casts that may be found in the urine smear.

Stool Samples:

Stool samples were collected from each patient in clean, dry and wide mouth containers.⁽⁹⁾ These stool samples were used for bacteriological and parasitological examinations.

a. Bacteriological Examination:

Stool was cultured in tetrathionate broth, peptone water, sea-salt, Salmonella-Shigella agar (S-S agar), Mannitol Salt agar (MSA) and MacConkey medium and incubated at 37°C for 24 hrs. In the second day, the culture was examined and the stool which was cultured in Tetrathionate broth and Peptone water was cultured in S-S agar and the stool which was cultured in sea-salt was cultured in Thiosulfate-Citrate-Bile-Sucrose agar (TCBS). Bacterial biochemical reactions (TSI, IMViC, oxidase, urease, coagulase, catalase) and gram stain, were done also.^(9,10)

b. Parasitological Examination:

1. Two direct wet smears were done for each specimen, one with physiological saline and the other with lugol's iodine, then covered with cover slip and the slide was examined under 10x and 40x objective lenses. Direct wet smear (with physiological saline) is used primarily to detect motile protozoan trophozoites. Helminth eggs and/or larvae, protozoan cysts, and coccidian oocysts may also be seen on the wet smear, although these are more often detected after fecal concentration procedures.⁽¹¹⁾

2. Formalin-Ether centrifugal sedimentation method was done for each stool sample,^(9,12) to look for all protozoan cysts, coccidian oocyst (especially *Cryptosporidium parvum*), and helminthes eggs and/or larvae.⁽¹¹⁾

Other tests

Complete blood pictures (Hb, WBC; total and differential, RBC, and Platelet) were done by using Coulter AC.T, from Beckman Coulter, USA. Chest x-ray was done for them as well.

The Direct Agglutination Test (by Koninklijk Institute Voor De Tropen-Royal Tropical Institute/ Amsterdam) was carried using leishmania freeze-dried antigen with a parasite density of (5×10^7 parasite per ml).

Results

The study had included 132 patients with VL confirmed by DAT and bone marrow examination. Seventy eight out of

132(59.0%) patients were boys, and 54(40.9%) were girls. The age of patients ranged between 2 months and 12 years, mean age \pm SD (5.89 ± 4.57). Most of the sero-positive patients were less than two years of age (74.3%).

The complications associated with the course of VL sero-positive patients were studied, **Table-1**. Among 132 sero-positive patients, Diarrhea with or without vomiting, was found in 75 patients (56.8%), followed by urinary tract infection in 30(23.4%) of the patients, and bronchopneumonia in 10 patient (7.5%).

The main non-infectious complications were malnutrition in 86 (65.1%) of patients, followed by bleeding tendency in 18 patients (13.6%), **Table-1**. Forty six (34.8%) of sero-positive patients were well nourished, while 86(65.2%) were malnourished. High proportion of them had mild or moderate malnutrition (45.5%), while (19.7%) had severe malnutrition.

Table 1. Complications reported among visceral leishmaniasis patients

Symptom	No. of cases	%
Malnutrition	86	65.1
Gastroenteritis	75	56.8
Urinary tract infection	30	22.7
Bleeding	18	13.6
Bronchopneumonia	10	7.5
Septicemia	9	6.8
Oral thrush	6	4.5
Skin rash	6	4.5
Meningitis	1	0.7
Otitis media	1	0.7
Coma	1	0.7

Bacterial Complications:

Pathogenic bacteria were isolated from 41 patients out of 132 (31.0%). Pure culture was demonstrated in 36 patients while 5 patients had mixed infections; two isolates were demonstrated from each one. Higher rate of infection was reported in urinary tract, 30 patients (22.7%). Septicemia was diagnosed in (6.8%) of the patients. Meningitis and otitis media were found in (0.7%) for each, **Table -2**

Eight types of bacteria were demonstrated. *Klebsiella* is the commonest type (16 isolates) followed by *E. coli* and *Proteus* in (11 and 9 isolates), respectively. These three types of bacteria represented (78.2%) of total isolates. This study shows that the *Klebsiella* is the most common cause of septicemia and urinary tract infection associated with VL. It represents (34.7%) of total isolates,

Table 2. Types of bacterial complications in 41 patients of visceral leishmaniasis

Type of infection	No. of isolates	No. of positive patients	(%) from total positive patients (No. 41)	(%) from total examined (No.132)
Urinary tract infection	33	30	(73.2)	(22.7)
Septicemia	11	9	(22)	(6.8)
Meningitis	1	1	(2.4)	(0.7)
Otitis media	1	1	(2.4)	(0.7)
Total	46	41	(100)	(31.0)

Table-3. *Escherichia coli* (*E. coli*) and *Proteus* also represent an important cause of urinary tract infections. Among 33 isolates from urinary tract, 10 isolates were *E. coli* and 9 were *Proteus*. These represent (21.7% and 19.6%) of total bacterial isolates that were demonstrated from various parts of the body, respectively.

Enterococcus faecalis was isolated from 3 patients of septicemia and 2 patients of urinary tract infection (6.5% and 4.3%) from total isolates, respectively.

Staphylococcus aureus, *Enterobacter*, *Pseudomonas aeruginosa* and *Neisseria*

meningitidis were also isolated but from patients of septicemia, urinary tract infection, otitis media and meningitis, respectively.

There was mixed infections in three patients of urinary tract infection; these are *E. coli* with *Proteus*, *Enterobacter* with *Klebsiella*, and *E. coli* with *Klebsiella*. Also there were another two patients who had mixed infection, one patient had urinary tract infection with *E. coli* and septicemia with *Klebsiella*, and other patient had urinary tract infection with *Proteus* and septicemia with *Enterococcus faecalis*, **Table-3.**

Table 3. Types of bacteria isolates from 41 complicated visceral leishmaniasis patients

Type of infection	Type of bacteria	In pure culture		In mixed culture		Total	
		No. of isolates	% from total isolates	No. of isolates	% from total isolates	No. of isolates	% from total isolates
Septicemia (total isolates: 11)	<i>Klebsiella</i>	4	(8.6)	1	(2.2)	5	(10.9)
	<i>Staph. aureus</i>	2	(4.3)	0	0	2	(4.3)
	<i>Enterococcus faecalis</i>	2	(4.3)	1	(2.1)	3	(6.5)
	<i>E.coli</i>	1	(2.2)	0	0	1	(2.2)
UTI (total isolates: 33)	<i>Klebsiella</i>	9	(19.5)	2	(4.3)	11	(23.9)
	<i>E.coli</i>	7	(15.2)	3	(6.5)	10	(21.7)
	<i>Proteus</i>	7	(15.2)	2	(4.3)	9	(19.6)
	<i>Enterococcus faecalis</i>	2	(4.3)	0	0	2	(4.3)
	<i>Enterobacter</i>	0	0	1	(2.2)	1	(2.2)
Meningitis (No.1)	<i>Neisseria meningitides</i>	1	2.2	0	0	1	2.2
Otitis media (No. 1)	<i>Pseudomonas aeruginosa</i>	1	2.2	0	0	1	2.2
Total	8	36	(78.3)	10	(21.7)	46	(100)

Parasitic Complications:

Parasitic infections were detected in 44 patients which represent (33.3%) of the total examined patients. The commonest

parasite detected was *Cryptosporidium parvum* (18.9%) followed by *Entamoeba histolytica* (13.6%). *Giardia lamblia*, *Blastocystis hominis*, *Hymenolypis nana*,

and *Enterobius vermicularis* were also detected but in lower frequency, **Table-4**. Fifteen patients had infection with mixed pathogenic parasites.

Non pathogenic parasites were also recovered from VL patients. Stool examination of these patients had revealed that (15.9%) of them have these parasites. The non pathogenic parasites that were recorded are; *Entamoeba coli* (7.6%), *Chilomastix mesnili* (4.5%), *Iodamoeba buetschlii* (3.8%), **Table-4**. *Monillia* which

is a fungus was also recovered in 11 cases (8.3%). Also the results of stool examination show that 14 patients (10.6%) harbored mixed infections with pathogenic and non pathogenic parasites.

Moreover, this study had revealed that 24 patients (18.1%) had mixed bacterial and parasitic infections. Twenty patients (15.1%) with bacterial urinary tract infection also have parasitic infection, while 4 (3.03%) patients with septicemia have parasites in their stools.

Table 4. Pathogenic and non-pathogenic parasites isolated from stool of visceral leishmaniasis patients

Type of parasite	No.	% of total examined
Pathogenic Parasites		
<i>C. Parvum</i>	25	(18.9)
<i>E. histolytica</i>	18	(13.6)
<i>G.lambliia</i>	5	(3.7)
<i>B.hominis</i>	6	(4.5)
<i>H.nana</i>	3	(2.2)
<i>E.vermicularis</i>	2	(1.5)
Non-pathogenic parasites		
<i>E.coli</i>	10	(7.6)
<i>C.mesnili</i>	6	(4.5)
<i>I.buetschlii</i>	5	(3.8)

Discussion

Leishmaniasis is recognized as an important public health problem in some member States of the Eastern Mediterranean region owing to its considerable impact on morbidity and its potential to spread in epidemics, which impose a heavy burden on national health services. ⁽¹³⁾

It was found that (56.1%) of the patients had malnutrition at the time of admission to hospital. These patients may be already malnourished before the onset of the disease; other factors include poor appetite of patients or the presence of other infections like diarrhea. Other study in Iraq recorded malnutrition cases in (81%) of the patients. ⁽¹⁴⁾ In Bangladesh, malnutrition was found in (83.3%) of VL cases. ⁽¹⁵⁾ However, Queiroz *et al* have reported that

approximately 27% of VL cases were severely malnourished. ⁽¹⁶⁾

It was mentioned that malnutrition is a risk factor for the development of VL. However, the immunological basis for this susceptibility is unknown. ⁽¹⁷⁾ At the same time it could be a consequence of the disease. Children are said to be at greater risk of developing VL when they are younger and more malnourished. If malnutrition really is associated with VL, this potentially fatal and visible disease may be a general indicator of community health among the rural and suburban poor. ⁽¹⁸⁾

Diarrhea with or without vomiting was recorded in (53%) of the patients. Pathogenic parasites were detected in (33.3%) of these patients. Bacterial causes for diarrhea were not demonstrated in this study including *Vibrio cholera*; other workers reported this type of diarrhea. ^(14, 19)

The frequency of bacterial infections in VL patients in the current study was (31%). There are many reasons for this incidence of bacterial infections in patients with VL. Malnutrition and leukopenia are two reasons for these complications. Other immunological factors include; depression of cell-mediated immunity due to *Leishmania* antigens and other non-related antigens,^(20,21) presence of serum suppression factors and nonspecific polyclonal B-cell activation with autoantibody production due to predominant Th2 cells activation in comparison with Th1 cells activation and the presence of high levels of immune complexes.⁽²²⁾

Queiroz *et al* in Brazil have found that infection was present in 10.9% of the patients at the time of admission, developed in 24.4% of patients during hospitalization.⁽¹⁶⁾ Higher percentages were reported by other workers in Iran; 41% reported by Kadviior,⁽²³⁾ and 42% by Barati *et al* ⁽²⁴⁾.

Urinary tract infection was the most common type of bacterial complication (21.9%). Other infections include lower respiratory tract infection (bronchopneumonia) in (7.5%) and septicemia (6.8%). The most common infections were pneumonia, otitis media, skin infections and sepsis.⁽¹⁶⁾

Barati *et al* have reported that urinary tract infections (seen in 36% of the patients with bacterial infection) were the most common, followed by blood (28%), respiratory (16%), gastro-intestinal (12%) and skin infections (8%).⁽²⁴⁾ However, Kadviior, reported that respiratory tract infection and septicemia were the most common types of bacterial infection (18.5% and 13.0%) respectively.⁽²³⁾

Both gram positive and gram negative bacteria were isolated from various samples, mostly are *Enterobacteriaceae* 38 (28.7%), followed by gram positive cocci 7(5.3%). Kadviior found that the most commonly pathogenic bacteria was *Enterobacteriaceae* (50%) in his study.⁽²³⁾ The leucopenia and immunosuppression

associated with VL probably facilitate the development of bacterial co-infections.⁽²⁴⁾

The incidence of pathogenic parasitic infections associated with the VL patients was (33.3%). *Cryptosporidium parvum* is the most common parasite that was detected in VL patients (14.9%), followed by *Entamoeba histolytica* (13.6%), and then the other types of pathogenic parasites but in lower rates. Malnutrition may be an important factor in the determining the increase in the frequency and severity of infectious illness.⁽²⁵⁾

Bleeding was observed in (13.6%) of the patients which is a sign of terminal stage of VL and it was the main cause of the death in 6 (40%) out of 15 cases died during the study period. Queiroz *et al* have reported that hemorrhagic phenomena were observed in 12.3% of the patients at the time of admission and in around 60% of the patients who died. It is, therefore, an important warning sign as to the severity of the disease.⁽¹⁶⁾

Oral thrush had been observed in (4.5%) of the patients, it is due to chronicity of the infection and depressed immunity, this findings is in agreement with study done in Missan governorate, Iraq.⁽¹⁴⁾

Skin rash other than bleeding tendency was observed in (4.5%) of the patients, while no dermal infection was reported in Missan study.⁽¹⁴⁾

From this study we conclude that bacterial infections and pathogenic parasites were confirmed in about one third of VL patients for each. A combination of approaches is important to control leishmaniasis: early recognition and treatment of patients and, where necessary, control of vectors and reservoir hosts. Health education of the population in endemic foci is the most important element of the control strategy.

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