Visceral Leishmaniasis Complicated By Secondary Bacterial Infections in Iraqi Kala-azar

Mohammed F. Al-Timmimi*, Sami Y. Guirges * , Abdul Rahman A. Al-Tae **

ABSTRACT:

BACKGROUND:

Kala-azar is a vector borne parasitic disease endemic in Iraq. This disease is complicated by secondary bacterial infections which may lead to death.

OBJECTIVE:

The study was carried out to detect the bacterial infections associated with kala-azar and the effective treatment.

MATERIALS AND METHODS:

Collection of blood, urine, stool and ear exudate specimens from 63 proved kala-azar patients. The bacterial isolates from the specimens were subjected to antibiotics sensitivity test.

RESULTS:

63 (46.7%) of 135 hospitalized children with visceral leishmaniasis, developing 102 episodes of infections. The sites of these infections were urinary tract 46(45.1%), lower respiratory tract 37(36.3%), gastrointestinal 10(9.8%) and middle ear 9(8.9%). Both Gram negative and Gram positive bacteria were isolated. Most of the isolated bacteria belong to the family *Enterobacteriaceae*. The antibiotics gentamicin, amikacin and co-trimoxazole were the most effective.

CONCLUSION:

Bacterial infections were common among hospitalized children with kala-azar. The commonest were Gram negative bacteria of the family *Enterobacteriaceae*.

KEY WORDS: Kala-azar , secondary bacterial infection, antibiotic sensitivity.

INTRODUCTION:

Leishmania infections are worldwide in distribution with major public health importance and considerable impact on their morbidity rate. Kala-azar which is caused by *Leishmania infantum* and of Mediterranean type endemic in Iraq [1,5].

The most common complications of visceral leishmaniasis (VL) leading to death including bleeding and bacterial superinfections, result from a decrease in blood elements due to leishmaniasis of the bone marrow and hypersplenism [6].

The present study is planned to investigate the secondary bacterial infections in patients with VL by cultivation and identification of bacteria isolated from blood, urine, stool and ear exudates and to find a suitable antimicrobial agents against the isolated microorganisms.

MATERIALS AND METHODS:

Two hundred fifty two specimens were collected from 63 hospitalized patients, their ages ranged from 4 months to 4 years. The specimens collected including blood, urine, stool and ear exudates from 63 out of 135 children whom they were diagnosed by immunochroma-tography and positive bone marrow smears for *Leishman Donovan* (L.D.) bodies in Al-Ilwyia Children Hospital and Ibn Al-Balady Maternity and Children Hospital in Baghdad. The processing of the collected speicmens were described below.

Specimens Collection and Isolation Procedures:

Blood Culture Procedure:

Blood were collected from infants and children (about 1-2ml) and mixed with 10 times its volume of brain heart infusion broth (2ml in 20ml of broth) and processed in the laboratory of Al-Ilwyia Children Hospital. Blood culture bottles were incubated at 37°C and routinely inspected twice a day for signs of microbial growth. The growth was evidenced by haemolysis, production of gas, coagulation of the broth, turbidity and a flocular deposit on the top of blood layer. When visible growth appears, Gram stained smear were prepared and examined for any microorganisms. If bacteria seen in the Gram stained smear, blood agar, chocolate agar under 5-10 percent carbon dioxide atmosphere and MacConkey agar plates were inoculated and incubated at 37°C for overnight and examined for microbial growth.

*AL- Llwyia Children Hospital, Baghdad, Iraq
**Dep, of Microblogy, Collegeof Medicine University of Baghdad.

Urine Culture Procedure:

Urine specimens were collected in sterile screw-capped bottles or urine bag for infants and neonates. A calibrated loop was applied to deliver a known volume of urine specimen when handled properly, to estimate the number of organisms in the original urine specimen based on colony forming unit (CFU) of bacterial growth on culture media. The calibrated inoculating loop was flamed and allowed to cool without touching any surface, urine specimen which was well mixed and the loop inserted into urine vertically to allow its adherence to the inoculating loop and a loopful of urine was streaked over the surface of MacConkey and blood agar plates. The plates were incubated at 37°C for 24 hours and colonies on each plate were counted and the number of CFUs was multiplied by 100 (a 0.01 ml loop was used) to determine the number of microorganisms per milliliter in the urine tested [7,8].

Stool Culture Procedure:

Stools were collected from children and rectal swabs from infants. Inoculation were made on enriched media, sodium tetrathionate broth and nutrient broth and incubated at 37°C for overnight to enhance recovery of enteric pathogens. MacConkey agar and salmonella – shigella agar plates were subcultured and incubated at 37°C for 24 hours for *Escherichia coli* and non-lactose fermentig shigella and salmonella. Non-lactose fermentig colonies from MacConkey agar plate were inoculated into urea agar slant, incubated at 37°C for 24 hours, then subculturing from each of negative urease to Kligler iron agar slant and incubated at 37°C for 24 hours, isolates were identified by agglutination tests with specific antisera.

Ear Swab Culture Procedure:

Cotton-tiped swabs were used to collect exudates or pus of middle ear and inoculated on blood agar, MacConkey agar, chocolate agar and Sabouraud dextrose agar. Blood and chocolate agar plates were incubated at 37°C under 5-10% carbon dioxide atmosphere and the MacConkey agar plate was incubated in air at 37°C for 24 hours. The Sabouraud agar plates were incubated at 26-28°C for at least 3 days or more to isolate if any monilia or fungi may be present in the ear exudate.

Identification of Isolated Microorganisms:

The bacterial isolates were identified by Gram's stained, colony appearance and according to their biochemical characteristics including IMVIC test, urease, oxidase, catalase, coagulase or any other necessary biochemical tests.

Due to the difficulty in obtaining reliable sputum sample from infants and small children for diagnosis of pneumonia, lower respiratory tract infections were evident by clinical manifestations and chest X-ray.

The antimicrobial susceptibility test of isolated microorganisms was carried out according to modified Kirby-Bauer disc diffusion method using different single antimicrobial discs [9,7].

RESULTS:

The incidence and sites of secondary bacterial infections associated with VL were demonstrated in Table (1). It was found that in 63(46.7%) of the 135 hospitalized children with VL, with a total of 102 episodes of infections. Urinary tract 46(45.1%), lower respiratory tract 37(36.3%), gastrointestinal 10(9.8%) and middle ear 9(8.8%) were the most common sites of bacterial infections in the present study.

Table 1: Sites of bacterial infections in 63 hospitalized children with VL.

Site of bacterial infections	Bacterial episodes	
	No.	%
Urinary tract	46	45.1
Lower respiratory tract	37	36.3
Gastrointestinal tract	10	9.8
Middle ear	9	8.8
Total	102	100

On the other hand, to investigate the types of secondary bacterial infections in children with VL and bacteriologic studies, it was found that out of the 135 hospitalized children with VL, 63(46.7%) had positive bacterial cultures and 72(53.3%) had cultures that showed no growth. Table (2) demonstrates the variety of infecting agents recovered from different sites of VL patients causing common bacterial infections, these were urinary tract infections, pneumonia, gastroenteritis and acute otitis media.

Table 2: Types of infection and frequency of bacteria recovered from 63 hospitalized children with VL.

Microorganisms	Urinary tract infection	Pneumonia	Gastroenteritis	Otitis media	Total No. (%)
Escherichia coli	27	0	6	0	33 (32.4)
Pseudomonas aeruginosa	7	14	0	0	21 (20.6)
Proteus mirabilis	8	0	0	0	8 (7.8)
Klebsiella pneumoniae	0	7	0	0	7 (6.9)
Serratia marcescens	4	0	0	0	4 (3.9)
Shigella Flexneri	0	0	4	0	4 (3.9)
Staphylococcus aureus	0	11	0	3	14 (13.7)
Streptococcus pneumoniae	0	5	0	6	11 (10.8)
Total No. (%)	46 (45.1)	37 (36.3)	10 (9.8)	9 (8.8)	102 (100)

The different bacterial species that were isolated and their frequency in pure and mixed cultures were shown in Table (3). From the data presented in this table it can be seen that the Gram negative bacilli constituted 77 isolates which were more frequently isolated than Gram positive cocci (25 isolates), and only 59 isolates (57.9%) of the Gram negative bacilli and 19 isolates (18.6%) of the Gram positive cocci were isolated in pure cultures.

Table 3: Bacterial profiles of 102 isolates recovered from 63 hospitalized children with VL.

Isolated bacteria	In pure culture		In mixed culture		Total	
	No.	%	No.	%	No.	%
Escherichia coli	27	26.5	6	5.9	33	32.4
Pseudomonas aeruginosa	13	12.8	8	7.8	21	20.6
Proteus mirabilis	8	7.8	0	0	8	7.8
Klebsiella pneumoniae	7	6.9	0	0	7	6.9
Serratia marcescens	0	0	4	3.9	4	3.9
Shigella flexneri	4	3.9	0	0	4	3.9
Total Gram negative isolates	59	57.9	18	17.6	77	75.5
Staphylococcus aureus	11	10.8	3	2.9	14	13.7
Streptococcus pneumoniae	8	7.8	3	2.9	11	10.8
Total Gram positive isolates	19	18.6	6	5.9	25	24.5
Total number of isolates	78	76.5	24	23.5	102	100

On the other hand, in analysing the pattern of mixed cultures we found that, from the 63 hospitalized children with VL, a total of 102 positive bacterial cultures were obtained; 24 cultures showed the growth of multiple organisms. The specific isolates in each mixed group were listed in Table (4). By inspecting the data presented in this table, it was found that 18 cultures (75.0%) revealed the growth of multiple Gram negative organisms, only 4 isolates (16.7%) showed the growth of mixed Gram positive and Gram negative organisms and 2 isolates (8.2%) revealed the growth of mixed cultures showing only Gram positive organisms.

Table 4: Distribution of bacterial isolates in mixed cultures recovered from 63 hospitalized children with VL.

Specific bacterial isolates	No. of mixed cultures	%
Esch. coli + Ps. aeruginosa	10	41.7
Ps. aeruginosa + Serratia marcescens	4	16.7
Serratia marcescens + Esch. coli	4	16.7
Ps. aeruginosa + Staph. aureus	4	16.7
Staph. aureus + Strept. pneumoniae	2	8.2
Total	24	100

In vitro sensitivities of various bacterial isolates from 63 hospitalized children with VL to 14 antimicrobial agents and the three most effective antibiotics against most frequent bacterial isolates were presented in Table (5). The antimicrobial agents applied for both Gram negative and Gram positive organisms were ampicillin, amikacin, cefalexin, cefotaxime, ceftazidime, co-trimoxazole, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, nitrofurantoin, piperacillin, tobramycin and vancomycin.

Table 5:The three most effective antibiotics against most frequent bacterial isolates recovered from 63 hospitalized children with VL.

Bacterial isolates	Effective antibiotics
Escherichia coli	Amikacin , cefotaxime and ciprofloxacine (96.9% for each).
Pseudomonas aeruginosa	Amikacin, gentamicin, & piperacillin (95.2% for each).
Proteus mirabilis	Cefotaxime, co-trimoxazole & nalidixic acid (87.5% for each).
Klebsiella pneumoniae	Amikacin, co-trimoxazole, cefotaxime (85.7% for each).
Serratia marcescens	Cefotaxime, nalidixic acid , & gentamicin (100% for each).
Shigella flexneri	Amikacin, cefotaxime, & nalidixic acid (100% for each).
Staphylococcus aureus	Amikacin, co-trimoxazole & vancomycin (92.8% for each).
Streptococcus pneumoniae	Amikacin , co-trimoxazole, & cefotaxime (81.8% for each).

DISCUSSION:

From the results obtained in the present study it was found that VL is complicated by secondary bacterial infections which represent the most common cause of death in this disease.

There are many reasons for a high incidence of bacterial infections in patients with VL. Malnutrition and leucopenia are two main reasons for complication of the disease. In VL patients, malnutrition functions as a predisposing factor and play a critical role in the progression to severe disease. The immunological factors which enhance the secondary bacterial infections associated with VL include:

- Depression of cell-mediated immunity due to *Leishmania* antigens and other non-related antigens [10,12].
- Presence of serum suppression factors capable of suppressing immune response.
- Non specific polyclonal B-cell activation with autoantibody production due to predominant Th2 cell activation in comparison with Th1 cell activation and the presence of high levels of immune complexes[13,14]. The incidence of bacterial infections in patients with VL in this study was 46.7%. In addition to high frequency of this complication in all patients with VL who died, bacterial infections where related to the cause of death. This finding is lower than the 52% reported by Guerreiro *et al* [15] and 60% found by Andrade *et al.* [16]. The lower frequency of bacterial infections in this study may be due to:
- The present study was considered bacterial infections only during hospitalization of clinically suspected VL patients.
- The use of some antibiotics before admission of clinically suspected VL patients.

In the present study UTIs, pneumonia, enteritis and otitis media were the most common types of bacterial infections associated with complicated VL. This finding was not in consistent with a study by other workers that reported the lower respiratory tract infections (Pneumonia) was the most common type of bacterial infection followed by urinary tract infection [13,15]. Another different study by Andrade *et al.*[16] found that the skin and the lower respiratory tract were the most common sites of bacterial infection associated with VL and these areas are also the most common sites of infections in immunocompromised hosts [17].

Both Gram positive and Gram negative bacteria were isolated from various samples in 63(46.7%) patients with complicated VL and most of these isolates were from the family *Enterobacteriaceae* 56(54.9%). This finding is in consistent with a study by Kadivar *et al.* [18] who reported that 7(50%) of the bacterial isolates belong to *Enterobacteriaceae*. The antibiotics given for VL patients complicated by bacterial infections were effective against both Gram positive and Gram negative bacteria and criteria based on antibiotic sensitivity results and the clinical experiences, gentamicin, amikacin, co-trimoxazole were the most commonly used antibiotics for all infants and young children with complicated VL for the coverage of common bacterial pathogens. This was in consistent with a study of Kadivar *et al.*[18] that recommended a combination of ampicillin and gentamicin for patients with VL for the coverage of common microorganisms. Another study by

Andrade *et al.*[16] reported that penicillin G and ampicillin were the most commonly useful drugs to

control bacterial infections in patients with complicated VL.

Antibiotic sensitivity testing was clinically beneficial for choosing the most suitable antibiotic to which the bacterial isolates were sensitive. The antibiotic susceptibility profile differs in accordance to the geographical area, time, environmental conditions and to the character of the pathogenic organisms.

The emergence of microorganisms with multiple drug resistance such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* renders the blood culture and antimicrobial sensitivity testing with new antibiotics a mandatory routine work.[19,20] Moreover, such a testing determine the antibiotics with good coverage against the commonly isolated bacteria in that particular period of time.

Since Gram negative bacteria constituted the majority 77(75.5%) of bacterial isolates recovered from VL patients, the aminoglycosides were the drug of choice in starting the empirical treatment till the culture and antibiotic sensitivity results were available for the clinician.

CONCLUSION:

Bacterial infections were common among hospitalized children with visceral leishmaniasis in Iraq. The commonest bacteria isolated were Gram negative bacteria of the family *Enterobacteriaceae*.

REFERENCES:

1. Taj-Eldin, S. and Al-Hassani, M.H. Kala-azar in Iraq: analysis of 100 cases. J. Fac. Med. Baghdad, 1961; 3:1-9.
2. Halawani, A. and Guirges, S.Y. Studies on the epidemiology of infantile kaka-azar in Iraq with special reference to leishmanin test. J. Egypt Publ. Health Ass. 1973; 48: 369-383.
3. Nouri, L. and Al-Jeboori, T.I. Kala-azar in Iraq: An epidemiological and clinical study. J. Fac. Med. Baghdad, 1973;15:72-85.
4. Rifaat, L.K. Comparison of serodiagnostic methods in kala-azar with special reference to immunoglobulin changes. M.Sc. Thesis, College of Medicine, Baghdad University, 1980.
5. Salih, S.M. Diagnostic, epidemiological study and evaluation of some immunological aspects in patients with visceral leishmaniasis in some regions of Iraq. Ph.D. Thesis, College of Science, University of Baghdad, 2006.
6. Peter, C.M. *Leishmania*. In: Behrman R.E., Kliegman, R.E. Jenson, H.B. Nelson Textbook of pediatrics. 16th ed. Philadelphia, WB Saunders, 2004:1130-33.
7. Vandepitte, J., Engback, K., Piot, P. and Heuck, C.C. Basic laboratory methods in clinical bacteriology. 1991, WHO, Geneva.
8. Forbes, B.A., Saham, D.F. and Weissfeld, A.S. Infections of the urinary tract. In: Diagnostic Microbiology. 10th ed., 1998, Mosby, U.S.A.
9. Bauer, Q.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. Antibiotic susceptibility test by a standardized single disc method. Am. J. Clin. Pathol. 1966: 45, 493-96.
10. Carvalho, E.M., Andrews, B.S., Martinelli, R. *et al.* Circulating immune complexes and rheumatoid factor in schistosomiasis and visceral leishmaniasis. Am. J. Trop. Med. Hyg., 1983; 32: 61-68.
11. Sacks, L.D., Lal, S,L., Srivastava, S.N., Blackwell J. and Neva, F.A. An analysis of T-cell responsiveness in Indian kala-azar. J. Immunol. 1987:138, 908-13.
12. Sundar, S., Reed, S.G., Singh, V.P., Kumar, P.C. and Murray, H.W. Rapid accurate field diagnosis of Indian visceral leishmaniasis. Lancet, 1998: 351, 363-65.
13. Galvao-Castero, B. *et al.* Polyclonal B-cell activation, circulating immune complexes and auto immunity in human American visceral leishmaniasis. Clin. Exp. Immunol. 1984:56: 58-66.
14. Abborzi, A., Torab-Jabromi, F. Auto-antibodies and complement levels of kala-azar patients in the south of Iran. Iran J. Med. Sci., 1993;18: 94-98.
15. Guerreiro, J., Ribeiro, S., Carvalho, E.M., Badaro, R. and Rocha, H. Infeccao bacteriana empacientes portadores de leishmaniose visceral (Bacterial infection in patients with visceral leishmaniasis). Mem. Inst. Oswaldo Guz. 1985;80, 447-50.
16. Andrade, T.M., Carvalho, E.M., and Rocha, H. Bacterial infections in patients with visceral leishmaniasis. J. Infect. Dis. 1990; 126:1354-59.
17. Young, L.S. Nosocomial infections in the immuno compromised adult. Am. J. Med. 1981;70,398-404.
18. Kadivar, M.R., Kajbaf, T.Z., Karimi, A. and Alborzi, A. Childhood visceral leishmaniasis complicated by bacterial infections. East. Meditt. Helth. J. 2000; 6, 879-83.
19. Bhattacharya, S.K., Sur, D. and Karbwang, J. Childhood visceral leishmaniasis. Ind. J. Med. Res. 2006; 123: 353-56.
20. Sipahi, T., Tavil, B. and Oksal, A. Visceral leishmaniasis and *Pseudomonas* septicemia associated with hemophagocytic syndrome and myelodysplasia in Turkish Child. Turkish J. Pead. 2005; 47: 191-94.