Visceral leishmaniasis in children: a cohort of 120 patients in a metropolitan city of Brazil

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There are few studies regarding the clinical presentation of visceral leishmaniasis (VL) in children. The aim of this study was to investigate the clinical manifestations, major complications and causes of death in children with VL.

A retrospective study was performed with pediatric patients (≤14 years old) with a diagnosis of VL in Fortaleza, state of Ceara, in Northeast Brazil.

A total of 120 patients were included. The mean age was 5±3.9 years, and 53.4% were male. The main clinical manifestations at admission were: fever (94.2%), splenomegaly (94.2%), hepatomegaly (82.5%), anorexia (55%), malaise (47.5%), cough (41.6%), abdominal pain (27.5%), vomiting (25.5%), and diarrhea (16.6%). Acute kidney injury was found in 25% of the patients. The main complication during hospital stay was pulmonary infection, found in 27.5% (n=33), leading to sepsis in 3 cases. Glucantime® was the drug of choice in 90% (n=108) of the cases, amphotericin B in 7.5% (n=9) and AmBisome® in 2.5% (n=3). Death occurred in 4 cases (3.3%) due to sepsis (3 cases) and hemorrhagic complications (1 case).

Visceral leishmaniasis is a frequent infection among children in our region. The main complications were pulmonary infection and acute kidney injury related to antiparasitic therapy, along with sepsis and hemorrhage.

Key words: visceral leishmaniasis, kala-azar, children, complications, mortality.

Visceral leishmaniasis (VL) or kala-azar is an endemic disease in tropical countries, subtropics, and southern Europe, affecting one to two million individuals and causing approximately 5,000 deaths each year[1-4]. It is estimated that over 200 million people around the world are at risk of acquiring the disease, and over 90% of the cases occur in five countries, including Brazil[5].

The majority of infected subjects have asymptomatic infection or present mild and unspecific symptoms such as diarrhea, dry cough, lethargy, low-grade fever, and hepatosplenomegaly[6]. As a consequence of the intense parasitism of the reticuloendothelial system, patients with VL present with accentuated anemia, leukopenia and thrombocytopenia, as well as with increased plasmatic levels of gamma globulins[6].

The prevalence of VL in the pediatric population has been documented in some studies[7,8] as due to the higher susceptibility to infections and the immune-depressed state found in this population, since the long-lasting immunity develops during those years[9]. In a recent study in our region, an important increase in the number of VL cases was observed, associated with urbanization of the disease[10]. Among 1379 registered cases in the study period, 493 (35.7%) were children[10].

The aim of this study was to investigate the clinical manifestations, major complications and causes of death in pediatric patients with VL.

Material and Methods

Study Subjects

A retrospective study was conducted of 120 consecutive pediatric patients aged ≤14 years, with confirmed diagnosis of VL, admitted to the São José Infectious Diseases Hospital, in Fortaleza, Northeast of Brazil, from December 2003 to December 2008. All patients had the diagnosis of VL based on the identification of amastigote in smears obtained from the posterior iliac crest or sternal bone marrow aspirate. Some patients also had rK39 antigen serum levels measured during active infection. A standardized case investigation form was used to complete demographical, epidemiological, clinical, and laboratory data.

The onset of symptoms was analyzed at the time of admission, and the mean length between the onset of symptoms and hospital admission, length of hospital stay, treatment, clinical complications, and mortality were recorded. Severity of disease was analyzed through clinical and laboratory findings. The clinical investigation included records of all clinical signs and symptoms presented by each patient at hospital admission. Laboratory data included an assessment of serum urea, creatinine, potassium, bilirubin, transaminases, lactate dehydrogenase, total blood count, prothrombin time, and urinalysis. Hemorrhage, manifested as gastrointestinal bleeding, hemoptysis and epistaxis, was recorded both at admission and during hospitalization.

The study protocol was reviewed and approved by the Ethical Committee of the Institution.

Definitions

Sepsis was defined according to the International Pediatric Sepsis Consensus Conference[11]. Anemia was graded as hemoglobin <11 g/dl for children <6 years old and <12 g/dl for children >6 years old. Oliguria was defined as a urine output <1 ml/kg/h in infants (1 to 12 months), and <0.5 ml/kg/h in children. We considered proteinuria and hemoglobinuria as one or more "+" in the qualitative exam. Nephrotic range proteinuria was defined as proteinuria exceeding 1000 mg/m^2 per day or spot (random) urinary protein-to-creatinine ratio exceeding 2 mg/mg. Hematuria was considered as one or more "+" in the qualitative exam and ≥1 erythrocytes per high power field. Leukocyturia was considered as the presence of ≥5 leukocytes per high power field. Respiratory insufficiency was defined as need of mechanical ventilation. Acute kidney injury (AKI) was defined according to pediatric RIFLE criteria (pRIFLE$_{\Delta\ SCr}$)[12], which uses the increase in serum creatinine (SCr) to define the groups. RIFLE criteria are defined by three grades of increasing severity of AKI – risk (Class R), injury (Class I) and Failure (class F) (Table I).

Statistical Analysis

The results were expressed through tables and summary measures (mean ± standard deviation) in the cases of quantitative variables. Statistical analysis was performed using software SPSS 10.0 (SPSS Inc. Chicago, IL, USA) and Epi Info, 6.04b, 2001 (Centers for Disease Control and Prevention, USA). Comparisons between quantitative variables were performed by Student's t test and between qualitative variables by Fisher's exact test. The descriptive values below 5% (p value <0.05) were considered statistically significant.

Results

There were 120 pediatric patients with a mean age of 5±3.9 years (range: 0 to 14 years), and 48% of them were under 2 years old. There were 64 (53.4%) males (Fig. 1). The mean length between the onset of symptoms and hospital admission was 38.5±40.4 days (range: 1 to 240 days). The average hospital stay was 20.4±8.2 days (range: 2 to 50 days).

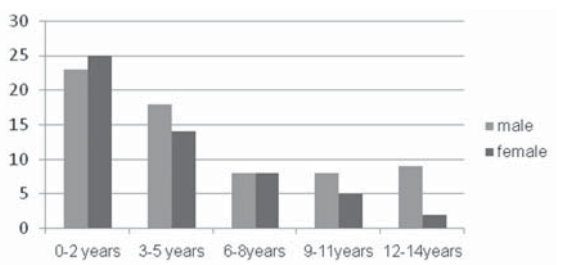

Fig 1. Distribution of pediatric patients with visceral leishmaniasis by sex and age at diagnosis.

Table I. Pediatric-Modified RIFLE ($pRIFLE_{\Delta SCr}$) Criteria

	SCr	Urine output
Risk	SCr increase by 25%	<0.5 ml/kg/h for 8 h
Injury	SCr increase by 50%	<0.5 ml/kg/h for 16 h
Failure	SCr increase by 75%	<0.3 ml/kg/h for 24 h or anuric for 12 h
Loss	Persistent failure >4 weeks	
End stage	End stage renal disease (persistent failure >3 months)	

SCr: Serum creatinine. pRIFLE: Pediatric risk, injury, failure, loss and end-stage renal disease.

The main clinical signs and symptoms present at admission were: fever (94.2%), splenomegaly (94.2%), hepatomegaly (82.5%), anorexia (55%), malaise (47.5%), cough (41.6%), abdominal pain (27.5%), vomiting (25.5%), and diarrhea (16.6%), as can be seen in Table II. Oliguria was found in 1.6% of the patients (n=2). Two patients had human immunodeficiency virus (HIV) infection. At presentation, 2 patients also had varicella, 1 had herpes zoster and 1 had dengue fever.

Acute kidney injury (AKI) was found in 30 patients (25%), and 24 (20%) had serum potassium <3.5 mEq/L. AKI patients were classified according to the $pRIFLE_{\Delta SCr}$ criteria, and 16 (53.3%) were found as Class R, 12 (40%) as Class I and 2 (6.7%) as Class F (p=0.004). Serum urea had significantly increased during the course of the disease (25±10 vs. 32±12 mg/dl, p<0.0001).

The laboratory analysis revealed the following: hemoglobin count 7.22±1.76 g/dl, white blood count 3570±1815/mm^3 and platelet count 89194±75724/mm^3; 57.5% had white blood count <3500/mm^3 and 25.8% had platelet count <50000/mm^3. All these parameters showed a significant increase from admission to discharge (7.2±1.7 vs. 9.0±2.0 g/dl, p<0.0001; 3570±1810 vs. 6437±9214/mm^3, p<0.0013; and 89194±75724 vs. 227129±108445/mm^3, p<0.0001, respectively). Serum albumin at admission was <3.5 g/dl in 59.1% of the patients and showed a significant increase from admission to discharge (2.98±0.62 vs. 3.57±0.56, p<0.0001). Aspartate aminotransferase (AST) at admission was 126.5±182.9 IU/L and alanine aminotransferase (ALT) was 67.7±85.7 IU/L. During the hospital stay, the AST and ALT levels were higher than 40 IU/L in 62.5% and 47.5% of the patients, respectively. Lactate dehydrogenase was 1316.3±1107.9 IU/L at admission, and it had significantly decreased from admission to discharge (1316.3±1107.9 vs. 504.8±285.2 IU/L, p=0.0004). The urinalysis found pH of 5.8±0.7; leukocyturia was found in 13 (10.8%) patients, proteinuria in 7 (5.8%) and hematuria in 6 (5%). The laboratory findings are shown in Tables III and IV.

The main complications during the hospital stay were secondary infections, which developed in 36 (30%) patients. Pneumonia was the major secondary infection, found in 25 (69.4%) cases. Other infections were: scabies (4 cases), otitis (2), phlebitis (2), cellulites (2), urinary tract infection (1), and impetigo (1). Three patients had pneumonia and sepsis. Hemorrhagic complications occurred in 1 case.

The drugs used for treatment of VL were Glucantime® in 108 (90%) cases, amphotericin B in 10 (8.3%) and AmBisome® in 2 (1.6%). Of the 108 patients using Glucantime®, 9 did not have a good response and the drug was replaced by amphotericin B. AKI was observed in 7 of 10 patients treated with amphotericin B, and according to the $pRIFLE_{\Delta SCr}$ criteria, 2 patients were classified as Risk, 4 as Injury and 1 as Failure.

Death occurred in 4 (3.3%) children; 3 had sepsis due to pulmonary infection and 1 had hemorrhagic complication. All children who died were under 1 year old.

Discussion

Human VL is an important endemic parasitic infection in Brazil. Although it is known as a rural disease, a high incidence of VL has been observed in large cities of Brazil due to favorable epidemiologic conditions and reduction of the natural environment of this zoonosis[10,13]. The consequence is an increase

Table II. Demographic Data and Clinical Manifestations of Children with Visceral Leishmaniasis (N = 120)

Age (years)	5 ± 3.9
Gender	
Male	64 (53.4%)
Female	56 (46.6%)
Hospital stay (days)	20±8
Signs and symptoms at admission	
Splenomegaly	113 (94.2%)
Fever	113 (94.2%)
Hepatomegaly	99 (82.5%)
Pallor	96 (80%)
Anorexia	66 (55%)
Weight loss	63 (52.5%)
Malaise	57 (47.5%)
Cough	50 (41.6%)
Vomiting	31 (25.8%)
Abdominal pain	33 (27.5%)
Diarrhea	20 (16.6%)
Chills	19 (15.8%)
Edema	18 (15%)
Lymphadenopathy	13 (10.8%)
Dyspnea	12 (10%)
Headache	10 (8.3%)
Jaundice	7 (5.8%)
Dehydration	4 (3.3%)
Crackles	2 (1.6%)
Epistaxis	2 (1.6%)
Oliguria	2 (1.6%)
Arthralgia	1 (0.8%)
Hematuria	1 (0.8%)
Malaise	1 (0.8%)
Myalgia	1 (0.8%)
Death	4 (3.3%)

in the number of VL cases in children younger than five years old, due to peri-domiciliary infection[8,13,14].

As found in other studies[8,14], the majority of children were under 6 years of age (66.7%) and a great number were under 2 years old (40%). Patients with HIV or acquired immunodeficiency syndrome (AIDS) are at greater risk of acquiring VL[15].

In the present study, the main clinical signs and symptoms presented in the initial evaluation were fever, splenomegaly, hepatomegaly, and pallor (in more than two-thirds of the cases), and anorexia, malaise and weight loss in half of the patients. These are the most characteristic clinical findings reported in VL[1,6,8,14,16-18].

According to recent studies, anemia is found in the majority of children with VL[8,13,18,19]. In our study, more than 90% of children had hemoglobin levels under 10 mg/dl. It is likely that the anemia has a multi-factorial origin, deriving from blockade of medullary production, splenic sequestration, immune hemolysis, hemorrhage, intestinal parasitosis, and iron deficiency[14]. In addition to anemia, other hematologic manifestations are usually present. In our study, half of the patients had white blood count lower than 3,500/mm^3, and one-fourth had platelet count lower than 50,000/mm^3. These findings are similar to other studies[8,13,19,20]. Thrombocytopenia, in many studies, is considered as a predictor factor for severe hemorrhage. In the present study, one patient died due to severe hemorrhage[8,19].

Liver involvement in VL is an indicator of severity. It is mostly found when the VL diagnosis is delayed. Mild forms of hepatitis are frequent and are usually diagnosed only by laboratory findings[14]. In our study, AST and ALT levels at admission were higher than 40 IU/L in 62.5% and 47.5% of the cases, respectively.

Secondary infection is one of the major complications found in children with leishmaniasis. This association was shown by Andrade et al.[17], who found a frequency of bacterial infection 4.8 times greater in patients who had been treated for VL when compared to children who had been treated for malnutrition in the same hospital. In a study made by Queiroz et al.[14], 10.9% of the patients had secondary infections at admission, 24.4% developed an infection during the hospital stay, and infection was present in 72.2% of the patients who died during the hospitalization. The main forms of infections were pneumonia, otitis, skin infections, and sepsis. In our study, secondary infections were found in 30% of the patients, and the main infections were also pneumonia, otitis and skin infection.

In the present study, AKI developed in a high number of patients receiving amphotericin B (33.3%). In a recent study with 48 patients

Table III. Comparison of Admission and Discharge Laboratory Data of Children with Visceral Leishmaniasis

	Admission	Discharge	P
U (mg/dl)	25 ± 10	26 ± 8	0.35
Cr (mg/dl)	0.5 ± 0.2	0.4 ± 0.2	0.3
Na (mEq/L)	133 ± 3.6	137 ± 3.7	<0.0001
K (mEq/L)	4 ± 0.6	4.2 ± 0.5	0.02
LDH (UI)	1316 ± 1107	504 ± 285	<0.001
Total bilirubin (g/dl)	1.5 ± 2.8	2.1 ± 3.5	0.59
Direct bilirubin (g/dl)	0.9 ± 2.2	1.2 ± 2.1	0.7
Indirect bilirubin (g/dl)	0.5 ± 1.2	0.9 ± 2.2	0.5
Albumin (g/dl)	3 ± 0.6	3.5 ± 0.5	<0.0001
AST (UI/L)	126 ± 183	90 ± 185	0.18
ALT (UI/L)	68 ± 86	62 ± 69	0.6
AP (UI/L)	309 ± 289	284 ± 143	0.77
Ht (%)	22 ± 5	28 ± 5	<0.0001
Hb (g/dl)	7.2 ± 1.7	9 ± 2	<0.0001
WBC (/mm^3)	3570 ± 1815	5638 ± 2070	<0.0001
Platelets (/mm^3)	89194 ± 75724	227129 ± 108445	<0.0001
HSR (mm/hr)	68 ± 34	54 ± 31	0.06
Prothrombin time (%)	60± 20	72 ± 19	<0.001

U: Urea. Cr: Creatinine. Na: Sodium. K: Potassium. LDH: Lactate dehydrogenase. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. AP: Alkaline phosphatase. Ht: Hematocrit. Hb: Hemoglobin. WBC: White blood cells. HSR: Hemosedimentation rate.

receiving amphotericin B, AKI (defined as an increase of more than 50% of the baseline creatinine) occurred in 31% of cases[21]. Higher incidences of AKI secondary to the use of amphotericin B have been reported, reaching levels as high as 80% of patients receiving this drug[22].

Incidence, prevalence and etiology of AKI in childhood are not yet well defined. A recent report from Houston, Texas, USA, stated that the most common causes of AKI in hospitalized children were renal ischemia (21%), pharmacologic agents (16%), and sepsis (11%)[23]. The true incidence of nephrotoxicity as a cause of AKI in children is unknown. In general, drugs are an infrequent cause of community-acquired AKI, especially in children. However, drugs and hypoxia are the leading etiologic factors for hospital-acquired AKI, and it remains a significant cause of morbidity and mortality in children[24]. Children with AKI caused by nephrotoxic agents have a significant risk for chronic renal injury. The mortality of children with AKI was found to be between 8% and 89% in retrospective studies[25-27]. In a prospective study, Bailey et al.[28] reported a mortality rate of 29.6% in patients with AKI compared with 2.3% in patients without AKI.

Despite its significant renal side effects, amphotericin B is still the drug of choice in the treatment of severe VL. Data on its nephrotoxicity and efficacy in the prevention are scarce. In retrospective studies, nephrotoxicity was found in 20–80% of adults and children[29-31]. There are two major hypotheses for the pathogenesis of amphotericin-B-induced AKI: (1) direct effects of the drug on ergosterol in the epithelial cell membranes and (2) renal vasoconstriction due to increased vascular resistance. Typically, intravenous administration results in an acute decrease of glomerular filtration rate (GFR) and transient oliguria followed by polyuria. Unlike most other drug-induced nephrotoxicity, most patients develop distal tubulopathy as well. The pharmacokinetics of amphotericin B in children appears to differ from that in adults because distribution volume is smaller and the clearance rate is faster. Risk factors for amphotericin-induced AKI include

Table IV. Laboratory Abnormalities in Children with Visceral Leishmaniasis

Laboratory findings	Patients with data	N (%)
Hb <10 g⁄dl	120	115 (95.8%)
WBC <3,000/mm^3	120	66 (55%)
Platelets < 80,000/mm^3	120	80 (66.6%)
AKI	120	30 (25%)
K <3.5 mEq/L	88	24 (27.3%)
Albumin <3.5 g/dl	104	78 (75%)
LDH >400 IU/L	38	32 (84.2%)
AST >40 IU⁄ L	100	85 (85%)
ALT >40 IU⁄ L	104	68 (65.4%)
Prothrombin time <60%	72	44 (61.1%)

Hb: Hemoglobin. WBC: White blood cells. AKI: Acute kidney injury. K: Potassium. LDH: Lactate dehydrogenase. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase.

cumulative dose, treatment duration and dosing schedule, concomitant therapy with diuretics or other nephrotoxic drugs, and impaired GFR at baseline.

Visceral leishmaniasis in children has a high lethality rate, around 10%[8,14,32]. In the present study, death occurred in 3.3% of cases, the majority of them caused by septic shock. All of the children who died were under 1 year old.

In conclusion, VL is a frequent infection among children in our region. The main complications were pulmonary infection and AKI related to antiparasitic therapy, along with sepsis and hemorrhage. Mortality was associated with age (<1 year), secondary infections and hemorrhagic complications.

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REFERENCES

1. Herwaldt BL. Leishmaniasis. Lancet 1999; 354: 1191-1199.
2. Efstratiadis G, Boura E, Giamalis P, et al. Renal involvement in a patient with visceral leishmaniasis. Nephrol Dial Transplant 2006; 21: 235-236.
3. Guerin PJ, Olliaro P, Sundar S, et al. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect Dis 2002; 2: 494-501.
4. Camargo LB, Langoni H. Impact of leishmaniasis on public health. J Venom Anim Toxins incl. Trop Dis 2006; 12: 527-548.
5. Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S. Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980-2004. Lancet Infect Dis 2005; 5: 763-774.
6. Salgado Filho N, Ferreira TM, Costa JM. Involvement of the renal function in patients with visceral leishmaniasis (kala-azar). Rev Soc Bras Med Trop 2003; 36: 217-221.
7. Correia JB. Epidemiology of visceral leishmaniasis in Pernambuco, north-east of Brazil and the use of a latex agglutination test in urine for its diagnosis [dissertation]. Liverpool: Liverpool School of Tropical Medicine; 1998.
8. Campos Jr D. Características do calazar na criança. Estudo de 75 casos. J Pediatr (Rio J) 1995; 71: 261-265.
9. Badaró R, Jones TC, Lourenço B. A prospective study of visceral leishmaniasis in an endemic area of Brazil. J Infect Dis 1986; 154: 639-649.
10. Albuquerque PL, Silva Junior GB, Freire CC, et al. Urbanization of visceral leishmaniasis (kala-azar) in Fortaleza, Ceará, Brazil. Rev Panam Salud Publica 2009; 26: 330-333.
11. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005; 6: 2-8.
12. Askenazi DJ, Bunchman TE. Pediatric acute kidney injury: the use of RIFLE criteria. Kidney Int 2007; 71: 963-964.

13. Cerf BJ, Jones TC, Badaro R, et al. Malnutrition as a risk factor for severe visceral leishmaniasis. J Infect Dis 1987; 156: 1030-1032.

14. Queiroz MJ, Alves JG, Correia JB. Visceral leishmaniasis: clinical and epidemiological features of children in an endemic area. J Pediatr (Rio J) 2004; 80: 141-146.

15. Daher EF, Fonseca PP, Gerhard ES, Silva Leitão TM, Silva Júnior GB. Clinical and epidemiological features of visceral leishmaniasis and HIV co-infection in fifteen patients from Brazil. J Parasitol 2009; 95: 652-655.

16. Boakye DA, Wilson MD, Kweku M. A review of leishmaniasis in West Africa. Ghana Med J 2005; 39: 94-97.

17. Andrade TM, Carvalho EM, Rocha H. Bacterial infectious in patients with visceral leishmaniasis. J Infect Dis 1990; 162: 1354-1359.

18. Alves JG. Calazar. In: Figueira F, Ferreira OS, Alves JG (eds). Pediatria - Instituto Materno Infantil de Pernambuco (2nd ed). Rio de Janeiro: Medsi; 1996: 320.

19. Nasir AM, Al-Nasser MN, Al-Jurayyan NA, et al. The haematological manifestations of visceral leishmaniasis in infancy and childhood. J Trop Pediatr 1995; 41: 143-148.

20. Berman JD. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 year. Clin Infect Dis 1997; 24: 684-703.

21. Berdichevski RH, Luis LB, Crestana L, Manero RC. Amphotericin B-related nephrotoxicity in low-risk patients. Braz J Infect Dis 2006; 10: 94-99.

22. Bagnis CI, Deray G. Amphotericin B nephrotoxicity. Saudi J Kidney Dis Transplant 2002; 13: 481-491.

23. Hui-Stickle S, Brewer ED, Goldstein SL. Pediatric ARF epidemiology at a tertiary care center from 1999 to 2001. Am J Kidney Dis 2005; 45: 96–101.

24. Patzer L. Nephrotoxicity as a cause of acute kidney injury in children. Pediatr Nephrol 2007; 23: 2159–2173.

25. Chan JC, Williams DM, Roth KS. Kidney failure in infants and children. Pediatr Rev 2002; 23: 47–60.

26. Andreoli SP. Acute renal failure. Curr Opin Pediatr 2002; 14: 183–188.

27. Moghal NE, Brocklebank JT, Meadow SR. A review of acute renal failure in children: incidence, etiology and outcome. Clin Nephrol 1998; 49: 91–95.

28. Bailey D, Phan V, Litalien C, et al. Risk factors of acute renal failure in critically ill children: a prospective descriptive epidemiological study. Pediatr Crit Care Med 2007; 8: 29–35.

29. Pathak A, Pien FD, Carvalho L. Amphotericin B use in a community hospital, with special emphasis on side effects. Clin Infect Dis 1998; 26: 334–338.

30. Harbarth S, Pestotnik SL, Lloyd JF, Burke JP, Samore MH. The epidemiology of nephrotoxicity associated with conventional amphotericin B therapy. Am J Med 2001; 111: 528–534.

31. Luber AD, Maa L, Lam M, Guglielmo BJ. Risk factors for amphotericin B-induced nephrotoxicity. J Antimicrob Chemother 1999; 43: 267–271.

32. Seaman J, Mercer AJ, Sondorp HE, Herwaldt BL. Epidemic visceral leishmaniasis in Southern Sudan: treatment of severely debilitated patients under wartime conditions and with limited resources. Ann Intern Med 1996; 124: 664-672.