Revista da Sociedade Brasileira de Medicina Tropical

REFERÊNCIA

BORGES, Myrlena Mescouto et al. Efficacy and safety of amphotericin B deoxycholate versus N-methylglucamine antimoniate in pediatric visceral leishmaniasis: an open-label, randomized, and controlled pilot trial in Brazil. **Revista da Sociedade Brasileira de Medicina Tropical**, Uberaba, v. 50, n. 1, p. 67-74, jan./fev. 2017. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0037-86822017000100067&lng=en&nrm=iso>. Acesso em: 22 fev. 2018. doi: http://dx.doi.org/10.1590/0037-8682-0455-2016.

Rev Soc Bras Med Trop 50(1):67-74, January-February, 2017
doi: 10.1590/0037-8682-0455-2016

Major Article

Efficacy and safety of amphotericin B deoxycholate versus N-methylglucamine antimoniate in pediatric visceral leishmaniasis: an open-label, randomized, and controlled pilot trial in Brazil

***Myrlena Mescouto Borges*[1],[2], *Maria Cristina da Silva Pranchevicius*[1], *Elza Ferreira Noronha*[2], *Gustavo Adolfo Sierra Romero*[2] *and César Omar Carranza-Tamayo*[2]**

[1]. Faculdade de Medicina, Universidade Federal do Tocantins. Palmas, TO, Brasil. [2]. Núcleo de Medicina Tropical, Universidade de Brasília. Brasília, DF, Brasil.

Abstract

Introduction: Despite their high toxicity, antimonials and amphotericin B deoxycholate are commonly used for treating visceral leishmaniasis (VL). Few studies showing conflictive data about their efficacy and adverse events in pediatric population are available. This study aimed to evaluate efficacy and safety of amphotericin B deoxycholate vs. that of N-methylglucamine antimoniate in treating pediatric VL in Brazil. **Methods**: This was a randomized, open-label, 2-arm and controlled pilot clinical trial. Treatment naïve children and adolescents with VL without signs of severe illness were treated with N-methylglucamine antimoniate (20mg/kg/day for 20 days) or amphotericin B deoxycholate (1mg/kg/day for 14 days). All patients were diagnosed with positive direct examination and/or positive PCR for *Leishmania* spp. performed in bone marrow samples. The primary efficacy end-point was VL cure determined after 180 days of completion of treatment. The analysis was performed using intention-to-treat (ITT) and per protocol (PP) analyses. **Results**: In total, 101 volunteers were assessed. Efficacy was similar for both groups. The antimonial (n=51) and amphotericin B groups (n=50) had a cure rate of 94.1% and 100%, and 94% and 97.9% according to ITT and PP analyses, respectively. All patients reported adverse events (AE). Serious AE incidence was similar in both groups. Five individuals were excluded from the study because of severe adverse events. **Conclusions**: N-methylglucamine antimoniate and amphotericin B deoxycholate have similar efficacy and adverse events rate in pediatric patients with VL.

Keywords: Visceral leishmaniasis. Children. Clinical trial. Brazil.

INTRODUCTION

Visceral leishmaniasis (VL) is a neglected infectious disease prevalent in children. In recent decades, its epidemiological features have changed in Brazil. In the past, VL was prevalent predominantly in rural environments; however, in recent years, it has been transmitted predominantly in urban and peri-urban areas[1]. Over the last decade, studies have increased the knowledge of diagnostic tools and efficacy of available leishmanicidal drugs. Nevertheless, clinical trials are scarce, especially among children[2,3].

According to Brazilian Ministry of Health, 3,453 individuals were diagnosed with VL in 2014, of which 52% were children and teenagers aged <19 years. Although 58.6% of VL cases occurred in Northeastern states, the state with the highest prevalence was Tocantins (10.8/100,000 population, more than 6 times the prevalence in Brazil in 2014, 1.7/100,000)[4].

Clinical features of VL mainly depend on the parasite-host relationship. Immunocompetent patients can resist parasitic infection, while parasite development is possible in anergic patients, resulting in typical systemic manifestations of the disease[5]. Splenomegaly and fever are the most important and common VL manifestations. Spleen size was found to be proportional with disease duration[6].

Drugs for VL have complex pharmacological properties. American VL needs parenteral administration of drugs with high toxicity levels[7]. Brazilian Ministry of Health suggested antimonials as first-line VL treatment and liposomal amphotericin B for patients at high mortality risk (infants, adults aged >50 years, immunosuppressed patients, patients with renal, hepatic or cardiac failure, etc.)[8]. Although drugs indicated for VL therapy in Brazil have been used for decades, few studies comparing their efficacy and safety in the pediatric population are available. Additionally, antimonial use in outpatient settings precludes detailed laboratory investigation of possible toxic effects and early identification of drug toxicity. Therefore, the

Corresponding author: Dr. César Carranza Tamayo.
e-mail: rhasec@unb.br
Received 26 October 2016
Accepted 7 February 2017

aim of this study was to estimate the efficacy and safety of amphotericin B deoxycholate (ABD) through a randomized and controlled clinical trial compared to meglumine antimoniate (MA) in children with VL treated in reference centers of Palmas (Tocantins' State Capital) and Araguaína, the two most populated cities in the State of Tocantins, Brazil.

METHODS

Study design and participants

This was an open-label, randomized, and controlled trial with masking laboratory outcomes. It was performed in Dona Regina Hospital in Palmas and Tropical Diseases Hospital in Araguaína city. Since analyzed drugs are marketed in Brazil, the survey was registered as a Phase IV study; however, it presented methodological characteristics of a phase III pilot clinical trial.

Eligible patients were children aged 6 months-12 years who met the inclusion criteria for VL diagnosis (presence of fever for at least two weeks associated with splenomegaly). The diagnosis of VL was confirmed by visualization of *Leishmania* spp. amastigotes in direct bone marrow microscopic examination or parasitic deoxyribonucleic acid (DNA) detection by polymerase chain reaction (PCR). Participation in this study was voluntary upon the signature of the informed consent form by the patients' parents or legal guardians. Exclusion criteria were the following: patients who underwent previous treatment with leishmanicidal drugs, clinically evident jaundice (total bilirubin > 2.5mg/dL), hemorrhages with coagulation disorders, generalized edema, signs of toxemia, severe malnutrition according to Gómez criteria[9], presence of comorbidities or immunosuppressive conditions, and lack of informed consent. Finally, 101 patients were included and underwent the randomization process between January 2007 and July 2009 (**Figure 1**).

Ethical consideration

This study attempted to continuously balance both individual and collective risks and benefits of the evaluated drugs used, always seeking maximum benefits and minimum risk or harm, and to avoid any damage, such as drug toxicity. The study was conducted strictly in accordance to the 196/96 Resolution of Brazilian Health Council. The parents or legal guardians of eligible patients received detailed explanation of the study protocol and provided written consent. The study protocol was approved by the Ethics Research Committee of the Federal University of Tocantins and complied with the tenets of the Declaration of Helisnki. All interventions were performed at no charge to the patients.

Interventions

Eligible patients underwent complete medical history and laboratory assessments. Samples were collected for complete blood count, human immunodeficiency virus (HIV) serology, prothrombin activity, bilirubin, aminotransferases, albumin, and globulin levels, and urinalysis. Blood and bone marrow samples were collected for VL diagnosis. Indirect immunofluorescence test (IFAT), rapid immunoassay (Kala-azar Detect®; InBios International, Seattle, WA), and standard polymerase chain reaction (PCR) for *Leishmania* spp. detection in the blood were performed. Bone marrow samples were obtained for direct microscopic examination to detect amastigotes of *Leishmania* spp., culture of *Leishmania* spp. in a biphasic medium, and PCR to amplify the conserved target region of 120bp of *Leishmania* kinetoplast deoxyribonucleic acid (kDNA)[10]. All patients underwent clinical and laboratory evaluation on days 0, 3, 7, 14, 21, 28, 60, 90, 120, 150, and 180.

Based on the randomization list, patients were administered either antimonial (antimonial group) or amphotericin B (ABD group). The treatment was administered using MA (Glucantime®, Aventis Pharma Ltda., Brazil) 20mg/kg/day IV for 20 days (antimonial group) and ABD 1mg/kg/day IV administered diluted in 5% glucose (concentration of 0.1mg/ml) infused for 6h for 14 days (ABD group).

All 4,500 ampoules of MA belonged to a single batch (603090, expiration date: Jun/2011). The 2,100 vials of ABD (Laboratório Cristália, Brazil) also belonged to the same batch (06085604, expiration date: Aug/2009). All drugs were provided by the Brazilian Ministry of Health.

Objectives

The primary objective was to evaluate the efficacy and safety of ABD compared to MA in children with visceral leishmaniasis. Secondary objective was to compare the time until clinical and hematological recovery between the groups.

Outcomes

Primary outcomes were clinical cure evaluated at day 180 of follow-up (D180) and prevalence of serious adverse events among patients, which required medication discontinuation and were excluded from the study.

Cure was defined as follows: complete remission of clinical signs and symptoms up to three months after treatment completion, normalization of hematological changes, and no recurrence of VL until the sixth month of follow-up. Recurrence was defined as the resurgence or reappearance of VL signs or symptoms after an improvement period or after clinical cure during the 6-month follow-up. Therapeutic failure was defined as any outcome different from cure. We included the interruption due to severe toxicity or drug intolerance, in the definition of therapeutic failure. In these cases, MA or ABD were replaced by liposomal amphotericin B.

Secondary outcomes included the following: time elapsed until fever resolution, time to 50% reduction of splenomegaly, time elapsed until normalization of RBC indices (hemoglobin and hematocrit), lethality rate, time elapsed until normalization of other laboratory parameters, and the overall rate of adverse events associated with drug use. The primarily analyzed adverse events were heart failure, kidney failure, liver failure, pancreatitis, and cardiac arrhythmias. Adverse events were defined and graded in accordance with the Division of AIDS Table for grading the severity of adult and pediatric adverse events[11].

Sample size

The original sample size was determined on the basis of the primary outcome endpoint of efficacy. To calculate sample size, an ideal setting with unlimited number of patients was

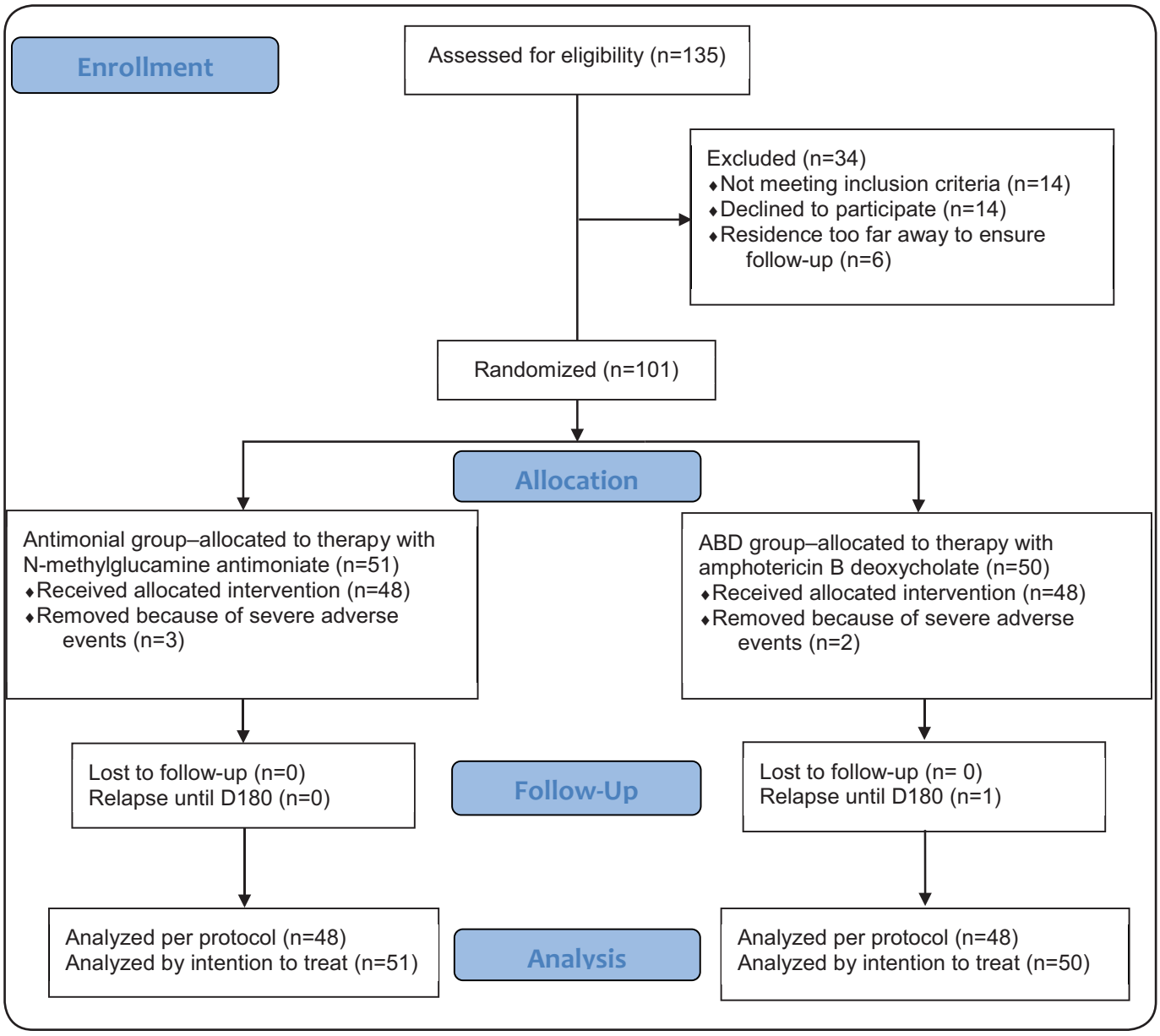

FIGURE 1 - Amphotericin B deoxycholate versus N-methylglucamine antimoniate: CONSORT (Consolidated Standards of Reporting Trials) **flow diagram.**

simulated and the formula suggested by Pocock was applied[12]. The required sample size was 283 subjects in each group. As a pilot trial, this study was projected with 50 individuals in each arm to evaluate the protocol.

Randomization

Randomization procedure was performed in blocks of 20, using Graphpad Quickcalcs free software (GraphPad Software Inc., San Diego, CA). This procedure was under the responsibility of an independent researcher from Tropical Medicine Center at *Universidade de Brasília*, who was not directly involved in any other operational aspect of the study. The names of compared drugs were typed on a sheet and placed in dark envelopes, which were sealed, stamped, and signed by those responsible for the randomization. The envelopes were opened immediately after the consent of the participants.

Blinding

This was an open-label trial. It was possible to mask the assessment of the hematological and biochemical tests. Laboratory technicians did not have access to patient's data, they had no direct contact with the investigators, and no information regarding the drug administered to the patients. No blinding was applied for the evaluation of clinical outcomes.

Statistical analysis

Statistical Package for Social Science (SPSS) version 18.0 (SPSS Inc., Chicago, IL) was used for systematization of clinical

and laboratory information. Data were analyzed with the same software. The two randomized groups were first compared with respect to baseline clinical and laboratory data. Proportions were compared through Yates' chi-square test. *T*-test for independent groups was used for continuous variables. Mann-Whitney U test was used for variables showing abnormal distributions. Differences were considered statistically significant at p-value < 0.05. The analysis of primary outcome was planned on an intention-to-treat basis (ITT) considering all subjects as originally assigned to the two arms. Patients who needed therapy change due to adverse effects and those lost during treatment or follow-up were considered as treatment failures. No subgroup analysis was initially planned.

RESULTS

The study was conducted between January 2006 and January 2009. A total of 135 children who met the suspect VL case definition as described above were screened and subsequently diagnosed with VL through laboratorial tests. Thirty-four children were initially excluded because of parents or legal guardians decline to participate (n=14), living far away from the study center (n=6), or not complying with the inclusion criteria (n=14). Of the recruited patients, 51 were randomly assigned to MA arm and 50 to ABD arm. Follow-up was completed by all participants in both arms (**Figure 1**).

Baseline data

The main baseline characteristics of the included children (101 patients) are reported in **Table 1**.

The median age was 38 months [range 6-143 months; 56 (55%) male] while children aged <5 and <2 years constituted 64.4% and 33.7% of the sample, respectively. Most patients were from the State of Tocantins, but 2 patients lived in the State of Pará.

The baseline characteristics of both arms were similar, with the exception of liver size measured below the right costal margin (median 4cm for antimonial group vs 5cm for ABD group; U=989, p=0.04) and spleen size measured below the left costal margin (median 6cm for antimonial group vs. 8cm for ABD group; U=878, p=0.02).

All patients included in the study had clinical features of VL. The main clinical signs on admission were fever in 100 (99%) patients, mucocutaneous pallor in 98 (97%), tachycardia in 76 (75.2%), hepatomegaly in 98 (97%), and splenomegaly in 101 (100%). Other signs and symptoms were vomiting in 48 (47.5%) patients, lethargy in 9 (8.9%), and localized edema in 3 (2.9%). Anemia (hemoglobin < 11g/dL), leucopenia (white blood cells - WBC < 5,000 cells/mm^3), or thrombocytopenia (platelets < 150,000/mm^3) were present in 89 (88.6%) patients. No patient had renal failure or hematuria at admission. Jaundice was observed in 4 children, but the aminotransferases and bilirubin levels 50% above the upper limit of normal occurred in 57% of patients. Twenty-two patients had serum albumin levels below 2g/dL.

The VL diagnosis was confirmed in all participants. Bone marrow direct examination was positive for 97 (96%) individuals. Four patients were diagnosed using PCR (one with positive indirect immunofluorescence test - IFI - and rapid test, and another with positive IFI).

Efficacy

Based on ITT analysis, cure was observed in 94.1% (48/51) of patients in antimonial group and in 94% (47/50) of patients in ABD group (p=0.62). Therefore, the observed difference in cure rate was 0.1% [95% confidence interval (CI) = -8.99-9.39] in the ITT analysis. The between-group difference in definitive cure rates in the ITT analysis was 0.1%, with 95% CI = -10.67-11.04. Most failures considered in the ITT analysis (5/6) were children who had to discontinue the therapy owing to serious adverse reactions and the remaining failure was due to relapse that occurred in one patient from ABD group in the sixth month of follow-up.

According to per protocol analysis, all (except one child from ABD group) patients who completed treatment were cured. In this analysis, 48 children treated with MA and 48 treated with ABD were included. The observed difference in cure rate was 2.1% with 95% CI= -5.52-10.9 (**Table 2**).

Secondary outcomes

The time elapsed until fever resolution was significantly shorter in antimonial group (22 children had complete remission in 48 hours), whereas eight patients in ABD group showed the same outcome ($\chi^2 = 8.91$, $p < 0.01$). Differences in reduction of spleen size were found between the groups: median spleen size at hospital discharge was 3 cm (50% reduction from admission spleen size) in antimonial group and 3.75cm (53% reduction from admission size) in ABD group (U=796.5, $p < 0.01$). The time elapsed until normalization of hemoglobin was evaluated at D60 and D90, and no difference was found between groups. In D60, 17 (34%) patients from antimonial group still had anemia (hemoglobin < 11g/dL), while 22 (43%) from ABD group remained with anemia. In D90, anemia was found in 8 (16%) children from antimonial group and in 10 (20%) patients from the ABD group ($\chi^2 = 0.09$, $p = 0.76$). No patient died during the study period. Time elapsed for normalization of other laboratory tests was evaluated at D90, and no differences were found between groups. Most patients (80%) showed normalization of hematological and biochemical indices in D90. The remainder persisted just with anemia.

Adverse events

All patients (in both groups) experienced at least one adverse event. Three serious adverse events occurred in antimonial group and 2 in ABD group, but no significant difference was found between the groups ($\chi^2 = 0.19$, $p = 0.98$). The main serious adverse events noted during hospitalization were abnormal liver enzymes, anemia, and hypomagnesemia (**Table 3**).

Patients in antimonial group had a higher frequency of serious adverse events requiring the withdrawal of research subjects. Most often adverse events observed in antimonial group were liver enzyme abnormalities (found in 18.8% of patients in D7, in 21.9% in D14, and in 17.6 % in D20), myalgia (n=32), anemia (severe in 37) and hypomagnesemia (severe in 24). Hypokalemia and hyponatremia events were considered

TABLE 1

Baseline clinical and laboratory data of patients with visceral leishmaniasis.

Variable	Antimonial group N-methylglucamine antimoniate (N= 51)	ABD group Amphotericin B deoxycholate (N= 50)
Age (months)	52.65	52.37
Male (%)	49	62.0
Weight (kg)	14.1	13.3
Fever (%)	94	88.0
Pallor (%)	96	100.0
Hepatomegaly (%)	96	100.0
Splenomegaly (%)	96	100.0
Associated bacterial infection (%)*	65	76.0
Spleen size: median of cm below the left costal margin	6	8
Liver size: median of cm below the right costal margin	4	5
Hemoglobin (g/dl)	7.81	7.67
White-cell count (per mm^3)	3,754	3.139
Platelet count (per mm^3)	142.780	117.509
Total bilirubin (mg/dl)	0.58	0.64
AST (IU/ml)	101.18	88.57
ALT (IU/ml)	69.4	69.31
Albumin (g/dl)	2.3	2.2
Globulin (g/dl)	3.7	3,8
Creatinine (mg/dl) **	0.495	0.493
Blood urea nitrogen (mg/dl) ***	21.44	17.22

* Bacterial infection was diagnosed on the basis of clinical suspect and/or positive cultures. ** To convert the values for creatinine to micromoles per liter, multiply by 88.4. *** To convert the values for blood urea nitrogen to micromoles per liter, multiply by 0.357.
ABD: amphotericin B deoxycholate; **AST:** aspartate aminotransferase; **ALT**: alanine aminotransferase.

TABLE 2

Amphotericin B deoxycholate versus N-methylglucamine antimoniate for pediatric visceral leishmaniasis treatment: efficacy data.

	Antimonial group N-methylglucamine antimoniate (N= 51)	ABD group Amphotericin B deoxycholate (N= 50)
Outcomes		
Early failure because of drug toxicity	3	2
Completed treatment	48	48
Relapse*	0	1
Lost to follow-up	0	0
Definitive cure at 6 months		
Intention-to-treat approach		
number of cured patients	48	47
percent (95% CI)	94.1 (84.1-97.9)	94.0 (83.8-97.9)
Per-protocol approach		
number of cured patients	48	47
percent (95% CI)	100 (92.5-100)	97.9 (89.1-99.6)

* Relapse was diagnosed in one boy at the final of follow-up (D180).
ABD: amphotericin B deoxycholate; **95% CI:** 95%confidence interval; **D180:** day 180.

TABLE 3

Safety data of Amphotericin B deoxycholate versus N-methylglucamine antimoniate for pediatric visceral leishmaniasis.

Event	Antimonial group N-methylglucamine antimoniate N= 51 (%)		ABD group Amphotericin B deoxycholate N= 50 (%)		P-value*
	moderate	severe	moderate	severe	
Abdominal pain (N/%)	2 (3.9)	-	5 (10.0)	-	0.42
Constipation (N/%)	1 (1.9)	-	-	-	0.99
Diarrhea (N/%)	2 (3.9)	-	2 (4.0)	-	0.62
Myalgia/arthralgia (N/%)	37 (72.5)	-	35 (70.0)	-	0.78
Nausea/vomiting (N/%)	29 (56.9)	-	35 (70.0)	-	0.24
Phlebitis (N/%)	1 (1.9)	-	3 (6.0)	-	0.61
Shivers (N/%)	1 (1.9)	-	10 (20.0)	-	0.01
Worsening anemia (N/%)	13 (21.5)	37 (72.5)	10 (20.0)	37 (74.0)	0.59
Worsening thrombocytopenia (N/%)	24 (47.1)	2 (3.9)	23 (46.0)	2 (4.0)	0.92
Abnormal alkaline phosphatase (N/%)	2 (3.9)	-	3 (6.0)	-	0.98
Abnormal ALT (N/%)	14 (27.5)	16 (31.3)	8 (16.0)	10 (20.0)	0.04
Abnormal AST (N/%)	20 (39.2)	8 (15.6)	10 (20.0)	6 (12.0)	0.03
Abnormal γ-glutamyl transpeptidase (N/%)	8 (15.6)	3 (5.8)	2 (4.0)	2 (4.0)	0.09
Abnormal total bilirubin (N/%)	1 (1.9)	-	1 (2.0)	-	0.48
Hypocalcemia (N/%)	8 (15.6)	2 (3.9)	6 (12.0)	2 (4.0)	0.79
Hypokalemia (N/%)	11 (21.6)	1 (1.9)	11 (22.0)	-	0.85
Hypomagnesemia (N/%)	15 (24.0)	24 (47.0)	19 (38.0)	20 (40.0)	0.85
Hyponatremia (N/%)	25 (49.0)	-	25 (50.0)	-	0.92

* P-value calculated by counting the total of moderate and severe adverse events described for each arm.
ABD: amphotericin B deoxycholate; **AST:** aspartate aminotransferase; **ALT**: alanine aminotransferase.

mild, but moderate hypocalcemia and severe hypocalcemia were observed in 8 and 2 children, respectively. Moderate hypomagnesaemia was observed in 15 patients, and 24 had very low magnesium levels. Gastrointestinal disorders such as nausea, vomiting, and abdominal pain were also reported. Cardiac arrhythmia was observed in 4 children from antimonial group, with no reported cases in ABD group (p = 0.13). Electrocardiographic changes associated with antimonial use were sinus arrhythmia associated with septal repolarization changes observed in 3 children and right branch conduction disturbance in one male child.

In the ABD group, increased frequency of anemia and hypomagnesaemia was found. Severe anemia was observed in 37 (74%) patients and 7 patients had moderate (n=5) or severe thrombocytopenia (n=2). Adverse events related to electrolytes were moderate hyponatremia, hypokalemia, and hypocalcemia, but magnesium levels dropped in 39 (78%) patients, with severe drop (below the minimum acceptable level) in 20 (40%) children. Ten children in ABD group reported shivers, and 2 maintained this complaint in D7. None of the patients had abnormal amylase, creatin kinase, creatinine, or urea levels. Other reported adverse events were changes in liver enzymes, gastrointestinal disorders (nausea, vomiting, abdominal pain, and constipation), myalgia, and arthralgia. Analysis showed that frequency of adverse events was similar between groups, except for abnormal aminotransferases levels (prevalent on antimonial group) and shivers (prevalent in ABD group).

DISCUSSION

Approximately two-thirds of the drugs used in pediatric clinical practice do not have enough evidence about dosages, safety, and efficacy for each phase of development[13]. Children compose a substantial proportion of the population of patients with VL, particularly in New World VL. They seemed to

have lower response rates to treatment, as well as recruitment difficulties in areas of high incidence of the disease and lack of diagnostic resources. To our knowledge, this is the first trial with focus on treatment with antimonial vs. ABD for pediatric visceral leishmaniasis in the New World. Both treatment groups were comparable at baseline by most of the recorded clinical and laboratory variables. Differences observed in the spleen size (2 cm larger in ABD group) and liver size (1cm larger in ABD group) had no clinical significance. In addition, both arms showed comparable efficacy, with no significant difference between groups.

The antimonial efficacy described in this study was high as per protocol analysis, but serious toxicity could have a negative impact on the efficacy, as indicated by the ITT analysis. It is described that sodium stibogluconate and N-methylglucamine antimoniate have comparable efficacy and toxicity[14]. Most of the studies that evaluate efficacy of antimonials for VL therapy are retrospective and performed in the Indian subcontinent. Average effectiveness of intramuscular antimonials assessed after six months without relapse was estimated in 92% of cases (range, 36-96%), based on previous studies[15]. Our study shows an efficacy comparable with that of previous studies. A Greek study described a 90.3% efficacy of using MA for 30 days in children[16]. However, since 1990s, increasing resistance to antimonials in Indian VL has been observed primarily in Bihar and adjacent territories. Randomized controlled trials with Indian patients with VL from 1992 to 2000 showed an efficacy of 36 to 69%, with an average of 50%[15-18]. As antimonials, ABD has been poorly evaluated in children with VL. Indian studies showed an efficacy ranging between 91.3 and 97% when compared to miltefosine[19,20]. Average effectiveness of ABD assessed after 6 months of follow-up was estimated at 97% (range 96-99%), based on six studies performed in the Indian subcontinent[15]. Our study showed a lower efficacy rate than that of previous studies. Lipidic formulations are considered comparable in efficacy, but with less toxicity[21]. Patients treated with antimonials had more effective response with the disappearance of fever during the first 48 hours, the opposite was observed in patients treated with ABD, in whom fever lasted longer than 72 hours. This observation is different from findings in the majority of previous studies comparing antimonials vs. amphotericin B[22]. Two studies performed in children with Mediterranean VL also showed earliest resolution of fever with amphotericin B[16,23]. Since this study had a limited population, this data should be reviewed in future studies with a larger sample group.

The toxicity observed in the present study was typical for used drugs and no unexpected adverse event was observed. Both drugs caused severe adverse events that precluded treatment continuation; this should be considered when planning the administration of antimonials to outpatients. Participants of the present study were treated after excluding signs and laboratory abnormalities commonly observed in severe cases. Serious adverse events observed in this trial can affect children without severe disease and can be fatal if managed in outpatient settings. Finally, although both treatments seemed to be similarly effective, ABD had the advantage of a shorter course.

Study limitations

Blinding was not possible owing to differences in formulations, time for treatment, and operational causes. This could have affected the assessment of efficacy, but we consider it unlikely, as the primary outcome was based on a long follow-up. The laboratory staff was blinded to the administered treatments. However, clinical side effects reporting may have been influenced both by the investigator's and the patients' parents or guardians knowledge of being research subjects. Results should be interpreted with caution because this study was a pilot trial, with a limited number of participants. Efficacy data reported cannot be extrapolated to children with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) or those with severe disease. On conclusion of this study, it was designed an open, controlled, randomized and multicenter clinical trial to evaluate the efficacy of four regimens for VL treatment in Brazil, including the liposomal formulation of amphotericin B (www.clinicaltrials.gov NCT01310738).

Conclusions and future directions

Both evaluated regimes (N-metilglucamine antimoniate and amphotericin B deoxycholate) used as first-line therapy in Brazil at the time the trial was conducted were effective with reasonable safety profile for most of the patients. However, serious adverse events occurrence suggest that high experienced clinical physicians are necessary for adequate management to avoid lethal outcomes. Toxicity profile was different between groups. Antimonial group had higher frequency of abnormal alanine transaminase (ALT) and aspartate transaminase (AST), while amphotericin group had higher frequency of shivers.

Results observed in both groups regarding cure rates suggest an equivalent effectiveness of tested regimens, with estimated difference of 2.1% and maximum inaccuracy rate of 10%. The appearance of relapses in a period over 6 months suggests the need to extend the monitoring period for 12 months after completion of therapy. The shorter course of treatment with amphotericin B should be considered an advantage over the antimonials treatment schedule of 20 days, mainly because the inpatient regimen is also recommended for antimonials. A pharmacokinetic study of antimonials in children would be particularly important to determine whether the relevant clinical effects could differ in accordance with age and the standard dose used.

Acknowledgments

We thank Dr. Erica Maués for collaborating on patient recruitment in Dona Regina Hospital.

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Werneck GL. Geographic spread of visceral leishmaniasis in Brazil. Cad Saúde Pública. 2010;26(4):644-5.

2. Kafetzis DA, Velissariou IM, Stabouli S, Mavrikou M, Delis D, Liapi G. Treatment of pediatric visceral leishmaniasis: amphotericin B or pentavalent antimony compounds? Int J Antimicrob Agents. 2005;25(1):26-30.

3. Romero GAS, Boelaert M. Control of visceral leishmaniasis in Latin America - A systematic review. PLoS Negl Trop Dis. 2010;4(1):e584. doi: 10.1371/journal.pntd.0000584.

4. Ministério da Saúde. Casos confirmados de Leishmaniose Visceral, Brasil, Grandes Regiões e Unidades Federadas. 1990 a 2014; Brasília: Ministério da Saúde; 2016. Disponível em: http://portalsaude.saude.gov.br/images/pdf/2016/maio/20/LV-Casos.pdf

5. Andrade ASS, Costa GC, de Aquino DMC, de Mendonça VRR, Barral A, Barral-Netto M, et al. Cytokines and visceral leishmaniasis: a comparison of plasma cytokine profiles between the clinical forms of visceral leishmaniasis. Mem Inst Oswaldo Cruz. 2012;107(6):735-9.

6. Braga ASC. Fatores associados à evolução clínica da leishmaniose visceral em crianças hospitalizadas em centro de referência de Belo Horizonte, 2001 a 2005. Dissertação de Mestrado. Belo Horizonte: Faculdade de Medicina. Universidade Federal de Minas Gerais; 2007. 98p.

7. Chávez-Fumagalli MA, Ribeiro TG, Castilho RO, Fernandes SOA, Cardoso VN, Coelho CSP, et al. New delivery systems for amphotericin B applied to the improvement of leishmaniasis treatment. Rev Soc Bras Med Trop. 2015;48(3):235-42.

8. Ministério da Saúde. MS amplia indicação de medicamento para o tratamento da leishmaniose visceral. Ministério da Saúde; 2014. Disponível em: http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/726-secretaria-svs/vigilancia-de-a-a-z/leishmaniose-visceral-lv/13058-ms-amplia-indicacao-de-medicamento-para-o-tratamento-da-leishmaniose-visceral

9. Gómez F, Ramos Galván R, Cravioto MJ. Studies on malnutrition in children. Bol Med Hosp Infant Mex. 1951;8(5):593-608.

10. Carranza-Tamayo CO, de Assis TS, Neri AT, Cupolillo E, Rabello A, Romero GA Prevalence of *Leishmania* infection in adult HIV/AIDS patients treated in a tertiary-level care center in Brasilia, Federal District, Brazil. Trans R Soc Trop Med Hyg. 2009;103(7):743-8.

11. National Institutes of Health (NIH). Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. Version 2.0. Bethseda, MD, USA: DAIDS; 2014. 33p.

12. Pocock SJ. The size of a clinical trial. *In:* Pocok SJ, editor. Clinical trials: a practical approach. Chichester, New York: John Wiley & Sons; 1983. p. 123-41.

13. Moreira MEL, Goldani MZ. A criança é o pai do homem: novos desafios para a área de saúde da criança. Cien Saúde Colet. 2010;15(2):321-7.

14. Chulay JD, Fleckenstein L, Smith DH. Pharmokinetics of antimony during treatment of visceral leishmaniaisis with sodium stibogluconate or meglumine antimoniate. Trans R Soc Trop Med Hyg. 1988;8(1)2:69-72.

15. Vanlerberghe V, Diap G, Guerin PJ, Meheus F, Gerstl S, Van der Stuyft P, et al. Drug policy for visceral leishmaniasis: a cost-effectiveness analysis. Trop Med Int Health. 2007;12(2):274-83.

16. Syriopoulou V, Daikos GL, Theodoridou M, Pavlopoulou I, Manolaki AG, Sereti E, et al. Two doses of a lipid formulation of amphotericin B for the treatment of Mediterranean visceral leishmaniasis. Clin Infect Dis. 2003;36(5):560-6.

17. Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-γ plus pentavalent antimony in Indian visceral leishmaniasis. J Infect Dis. 1997;176(4):1117-9.

18. Thakur CP, Kanyok TP, Pandey AK, Sinha GP, Messick C, Olliaro P. Treatment of visceral leishmaniasis with injectable paromomycin (aminosidine). An open-label randomized phase-II clinical study. Trans R Soc Trop Med Hyg. 2000;94(4):432-3.

19. Singh UK, Prasad R, Mishra OP, Jayswal BP. Miltefosine in children with visceral leishmaniasis: a prospective, multicentric, cross-sectional study. Indian J Pediatr. 2006;73(12):1077-80.

20. Sundar S, Jha TK, Thakur CP, Bhattacharya SK, Rai M. Oral miltefosine for the treatment of Indian visceral leishmaniasis. Trans R Soc Trop Med Hyg. 2006;100(suppl 1):S26-S33.

21. Falci DR, da Rosa FB, Pasqualotto AC. Comparison of nephrotoxicity associated to different lipid formulations of amphotericin B: a real-life study. Mycoses. 2015;58(2):104-12.

22. Mishra M, Biswas UK, Jha AM, Khan AB. Amphotericin versus sodium stibogluconate in first-line treatment of Indian kala-azar. Lancet. 1994; 344(8937):1599-1600.

23. Apa H, Devrim I, Bayram N, Deveci R, Demir - Ozek G, Carti OU. Liposomal amphotericin B versus pentavalent antimony salts for visceral *Leishmania* in children. Turk J Pediatr. 2013;55(4):378-83.