Revista da Sociedade Brasileira de Medicina Tropical 46(1):55-59, Jan-Feb, 2013
http://dx.doi.org/10.1590/0037-868216432013

Major Article

Factors of poor prognosis of visceral leishmaniasis among children under 12 years of age. A retrospective monocentric study in Belo Horizonte, State of Minas Gerais, Brazil, 2001-2005

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ABSTRACT

Introdution: A major concern with the visceral leishmaniasis (VL) is its high lethality rate, even with proper treatment. Low age, prior malnutrition, disease duration prior to diagnosis, severe anemia, fever for more than 60 days, diarrhea and jaundice are known poor prognostic factors. The goals of this study are to describe the clinical and laboratory characteristics of VL among children under 12 years of age and to identify the factors associated with VL poor outcome. **Methods:** Two hundred and fifty children under 12 years of age with confirmed VL admitted to Hospital João Paulo II (FHEMIG), Belo Horizonte, Brazil, between January 2001 and December 2005 were evaluated retrospectively. The primary outcome was the poor clinical evolution: sepsis, and/or pneumonia, and/or urinary tract infection, and/or of bleeding (expect epistaxis), and/or severe neutropenia (neutrophil < 500 cells/mm^3). Odds ratio (crude and adjusted) and its 95% confidence interval for each variable were calculated. Values less than 0.05 were considered significant. **Results:** Average age was 3.3 years (3.6 months-11.6 years), 71.2% were younger than 5 years and 47.2% lived in Metropolitan Area of Belo Horizonte. The mean fatality rate was 3.6%. Sixty-six (26.4%) patients presented poor evolution. After a multivariate analysis, age <18 months, abnormal respiratory physical examination on hospital admission, and platelets <85,000/mm^3 remained associated with increased chance of poor evolution. **Conclusions:** The results suggest that patients aged between 12 and 18 months, with platelet counts bellow 85,000/mm^3, and respiratory abnormalities at admission should be considered potentially severe.

Keywords: Visceral leishmaniasis. Children. Prognosis.

INTRODUCTION

The leishmaniasis, found in 88 countries in four continents, comprise one of the 10 global priority endemics of the World Health Organization. It is estimated that there are 500,000 new cases of visceral leishmaniasis (VL) and 50,000 deaths per year worldwide. Over 90% of VL cases occur in India, Bangladesh, Nepal, Sudan and Brazil[1-3].

In Brazil, VL occurs all over the country. It happens mostly in the Northeast region (approximately 70% of total cases), followed by the Southeast, North and Midwest regions. According to the Ministry of Health, from 2001 to 2010, 33,473 cases were reported in Brazil, with 2,267 deaths[4-6]. From the 1980s, it was observed that VL had spread to the urban areas, mainly to state Capitals, which contributed to the increase of occurences outside Northeast region and among children under 10 years of age (56.7% of total cases)[4,7]. In Belo Horizonte, 223 cases were reported between 1994 and 1999, and 42.8% of them among children younger than 10 years old[8].

One of the biggest concerns regarding VL is its high lethality rate, which reaches almost 100% among not treated symptomatic patients. Between 1-5% of treated individuals die due their resistance to chemotherapy, drug toxicity or diseases complications itself, especially in cases of late diagnosis[9]. In Brazil, the average lethality from 2000 to 2010 was 6.5%, with an increase of 93,8% between 2001 (3.2%) and 2010 (6.2%)[10]. In Belo Horizonte, in 2010, the fatality rate was 15.7%[8].

Some factors associated with disease poor prognosis are well established, such as young age, prior malnutrition, disease duration prior to diagnosis, as well as severe anemia, fever for more than 60 days, diarrhea and jaundice. The mortality in hospitalized patients is related to secondary infections such as pneumonia and sepsis, as well as hemorrhagic complications[11-13].

The objectives of this study are: describe clinical and laboratorial characteristics of VL among children under 12 years of age admitted to a reference center of peadiatrics infectious and parasitic diseases in Belo Horizonte, Minas Gerais, Brazil, and identify factors associated with worse evolution of VL at hospital admission.

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Received in 18/05/2012
Accepted in 09/10/2012

METHODS

This is a retrospective study conducted at *Hospital João Paulo II* (HJPII) of the *Fundação Hospitalar of Minas Gerais State* (FHEMIG), in the period from January 2001 to December 2005.

Our study has identified 259 children that have been diagnosed with VL by indirect immunofluorescence assay (IFA) and by parasites detection in bone marrow or other organs aspirate during the study period. Of these, nine were excluded: three because they had been treated at another hospital before admission in HJPII and six for being older than 12 years of age, which resulted in a final sample of 250 children. Epidemiological, demographic, symptoms and clinical signs (hepatomegaly, splenomegaly, fever) data, specific and supportive treatments, clinical evaluation and nonspecific laboratory tests (erythrocyte, leukocyte count and platelet counts, albumin and globulin levels) were recovered from patients' records.

The primary study endpoint was the clinical course of patients, divided into satisfactory and unsatisfactory. It was considered unsatisfactory evolution the occurrence of sepsis, and/or pneumonia, and/or urinary tract infection, and/or bleeding (except epistaxis), and/or severe neutropenia (neutrophil <500 cells/mm^3)[12-17]. Urinary tract infection was included in the criteria to avoid the potential co-intervention represented by the antibiotics use to treat it.

EpiInfo® MS-DOS® 6.04 and EpiInfo® for Windows® 3.4.3 (Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America) were used for data management and statistical analysis. The nutritional evaluation was carried out using Epinut application, with NCHS (National Center for Health Statistics) curve as reference. It was considered to be undernourished the patient with Z score (weight/age) of less than 2 standard deviations (SD).

The descriptive analysis was made through the distribution of frequency, mean and confidence interval of 95% (95%CI), according to the variable type. Variables with more than 30% of missing values were excluded from the multivariate analysis. Univariate analysis was performed between the primary endpoint and socio-demographic, clinical and laboratory pretreatment (hospital admission) data, using chi-square (x^2) with Yates correction or Fisher's exact test. We calculated the crude and adjusted odds ratios by logistic regression and 95%CI for each categorical variable. Variables with $p \leq 0.20$ were selected for multivariate analysis. In the final analysis, values less than 0.05 were considered significant.

Ethical considerations

This study is in accordance with Resolution 196/96 of the Brazilian National Health Committee and its updates, and it has been approved by the Ethics Committes in Research of *Centro de Pesquisas René Rachou*, *Fundação Oswaldo Cruz* (FIOCRUZ) and *Hospital João Paulo II*.

RESULTS

The average age of the 250 patients studied was 3.3 years (from 3.6 months to 11.6 years), with 71.2% of them being under the age of 5 years and 9.2% under 1 year. Nearly half of the children were male (52%). There was no difference regarding gender between the groups. The majority of patients lived in Belo Horizonte (39.6%) or in Metropolitan Area of Belo Horizonte (47.2%). We observed 17 cases in 2001, 49 in 2002, 50 in 2003, 74 in 2004 and 60 in 2005, with nine deaths in total. Mean lethality was 3.6%, ranging from zero in 2001 to 8% in 2003.

The time between onset of symptoms and hospitalization ranged from two to 300 days, with an average of 17.5 days. Symptoms began within 30 days before admission in 80% of patients. The average length of hospital stay was 15.4 days (SD $\pm$ 9.5), ranging from two to 81 days. 59.3% of patients, who did not die, were discharged in less than two weeks, and 32.4% in 10 days or less. Among the symptoms, the highlights were: fever (98.8%), appetite loss (52.8%), abdominal distension (44.8%), and weakness (42.4%). The most frequent signs at admission were: splenomegaly (98.4%), hepatomegaly (97.2%) and pallor (94%). The most frequent laboratory abnormalities were hematological: anemia (93.6%), thrombocytopenia (86.4%), leukopenia (78.4%), pancytopenia (66.8%) and neutropenia (33.6%). The hypergammaglobulinemia was present in 92.6% of cases, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly elevated in 90.3% and 69.3% of the patients, respectivelly. The prothrombin activity was less than 70% in 67.9% of cases and hypoalbuminemia was present in 88% of patients. Only 13.5% of patients had hyperbilirubinemia.

The average time to specific VL therapy beginning after hospitalization was 3.5 days. The N-methylglucamine was the initial treatment in 241 (96.4%) patients, with average administration time of 11.3 days (1-33 days). Only nine (3.6%) children started treatment with amphotericin B deoxycholate due to liver abnormalities (hyperbilirubinemia or elevated aminotransferases), renal abnormalities (kidney hematuria or high scorias), and less than one year of age. In 13 patients, N-methylgucamine was replaced by amphotericin B deoxycholate due to: respiratory and/or hemodynamic instability (10), treatment failure with N-methylgucamine (5), elevated aminotransferases or bilirubin, or coagulation abnormalities (6), prolonged corrected QT interval (3), increased renal scorias (1), and young age (1).

The average time between treatment start and fever regression was 5.6 days (SD$\pm$3.8 days), varying from one to 26 days. In less than seven days, 72.3% (154/213) of patients were afebrile in less than seven days.

Clinical complications during treatment were observed in 144 (57.6%) patients. Severe anemia was the most frequent occurence (78-31.2%) followed by: infection (58-23.2%), febrile neutropenia (55-22%), hemorrhagic complications (41-16.4%), bronchospasm (38-15.2%) and heart failure (12-4.8%). Two patients had liver failure, only one child had kidney

failure, and four had seizures. Additional clinical support was indicated for 55.6% (139) of children, especially the use of blood derivates (red blood cells: 78, plasma: 23, and platelets: 22), antimicrobial therapy (54 febrile neutropenic and 46 localized infections), bronchodilators (50) and diuretics (43). Fourteen patients required treatment in intensive care unit (ICU).

All nine patients who died during the follow-up period were admitted to the ICU. The mean time between hospitalization and death was 15 days (5-32 days). None presented Z score (weight/age) of less than 2SD. At admission, bleeding was observed among three patients, and edema among six. Three patients had jaundice and all had hepatosplenomegaly. Sepsis was reported in seven patients who died, eight had reference to shock on their death certificate, and three had reference to bleeding.

Most patients (n = 184, 73.6%) had a satisfactory evolution and 66 (26.4%) had unsatisfactory evolution. **Table 1** shows the frequency of clinical manifestations stratified according to evolution. In univariate analysis these were associated with unsatisfactory evolution: age <18 months, the presence of jaundice on physical examination, poor capillary

TABLE 1 - Socio-demographic, clinical and laboratory findings associated with unsatisfactory evolution of visceral leishmaniasis in 250 children admitted to* Hospital João Paulo II, *in Belo Horizonte, State of Minas Gerais, Brazil, 2001-2005.

			Unsatisfactory evolution			
Variable	**n**		**yes**	**no**	**p**	**OR**
Age <18 months	250	yes	23	27	0.0008	3.11
		no	43	157		
Being from Metropolitan Area of Belo Horizonte	250	yes	52	165	0.0424	0.43
		no	14	19		
Symptoms for more than 60 days	248	yes	8	16	0.5546	1.46
		no	57	167		
Jaundice on physical examination	223	yes	8	5	0.00728	5.12
		no	50	160		
Poor capillary perfusion	201	yes	3	0	0.9657	-
		no	52	146		
Abnormalities on respiratory examination	250	yes	10	4	0.00029	8.04
		no	56	180		
Abnormalities on cardiovascular examination	250	yes	22	51	0.4820	1.30
		no	44	133		
Liver enlargment > 4.5cm	250	yes	36	67	0.0154	2.10
		no	30	117		
Spleen enlargment > 6.5cm	248	yes	34	75	0.1513	1.58
		no	31	108		
Malnutrition (z score < - 2SD)		yes	8	14	0.3915	1.43
		no	58	170		
Hemoglobin < 9.0g/dl	250	yes	63	151	0.0141	4.59
		no	3	33		
Leukocytes < 3,500 cell/mm^3	250	yes	38	85	0.14901	1.58
		no	28	99		
Platelets < 85,000/mm^3	249	yes	44	74	0.0004	2.95
		no	22	109		
AST (> 185mg/dl)	226	yes	23	24	0.00041	3.44
		no	39	140		
ALT (> 105mg/dl)	225	yes	20	28	0.0136	2.30
		no	42	135		
Total bilirubin > 1.3mg/dl*	111	yes	12	3	0.00029	10.22
		no	27	69		
Prothrombin activity < 60%*	106	yes	30	23	0.01084	3.02
		no	16	37		
Albumin < 2.5g/dl*	158	yes	40	44	0.00000	10.30
		no	6	68		

OR: odds ratio; **SD**: standard deviation; **AST**: aminotransferase; **ALT:** alanine aminotransferase. *Variable eliminated from multivariate analysis because of high loss of information.

TABLE 2 - Multivariate analysis of factors associated with the unsatisfactory evolution of visceral leishmaniasis in 250 children admitted to Hospital João Paulo II in Belo Horizonte, State of Minas Gerais, Brazil, 2001-2005.

	Crude		Adjusted		
Variable	**OR**	**95%CI**	**OR**	**95%CI**	**P**
Age <18 months	3.11	1.53 to 6.31	4.20	1.61 to 10.98	**0.0034**
Being from Metropolitan Area of Belo Horizonte	0.43	0.19 to 0.98	0.34	0.11 to 0.98	**0.0460**
Jaundice on physical examination	5.12	1.42 to 19.22	1.88	0.43 to 8.21	NS
Abnormalities of respiratory examination	8.04	2.18 to 32.16	9.87	2.27 to 42.99	**0.0023**
Liver enlargment > 4.5cm	2.10	1.13 to 3.88	1.69	0.75 to 3.78	NS
Spleen enlargement > 6.5cm	1.58	0.86 to 2.92	1.08	0.47 to 2.46	NS
Hemoglobin < 9.0g/dl	4.59	1.27 to 19.73	5.09	0.92 to 28.25	NS
Leukocytes < 3,500 cell/mm^3	1.58	0.86 to 2.92	2.01	0.89 to 4.56	NS
Platelets < 85,000/mm^3	2.95	1.56 to 5.59	2.18	1.01 to 4.70	**0.0473**
AST (> 185mg/dl)	3.44	1.65 to 7.17	3.19	0.92 to 11.04	NS
ALT (> 105mg/dl)	2.30	1.11 to 4.76	1.07	0.31 to 3.68	NS

OR: odds ratio; **95%CI:** 95% confidence interval**;** NS: not significant; **AST**: aminotransferase; **ALT:** alanine aminotransferase.

perfusion, abnormalities of respiratory examination, liver enlargment > 4.5cm, hemoglobin <9.0mg/dl, leukocytes <3,500 cell/mm^3, platelets <85,000/mm^3, AST > 185mg/dl, ALT > 105mg/dl, total bilirubin > 1.3mg/dl,prothrombin activity < 60% and albumin < 2.5g/dl. Hemoglobin <7.0mg/dl was tested and were not association with unsatisfactory evaluation. Those who were coming from Metropolitan Area of Belo Horizonte had reduced risk of unsatisfactory evolution **(Table 1)**.

After the multivariate analysis **(Table 2),** these factors remained associated with increased odds of unsatisfactory evolution: age < 18 months (odds ratio; OR 4.20), abnormalities on respiratory examination (OR 9.87), and platelets < 85,000/mm^3 (OR 2.18). Being from Metropolitan Area of Belo Horizonte (OR 0.34) was the only factor with a protective effect on poor evolution in this population **(Table 2)**.

DISCUSSION

The number of children hospitalized at *Hospital João Paulo II, Fundação Hospitalar do Estado de Minas Gerais* corresponded to approximately 25% of total VL cases reported in Minas Gerais and 63.5% of the cases in children under 14 years reported in Belo Horizonte during the study period.

In terms of age, children under 18 months of life had a worse prognosis. Other authors observed increased risk of poor evolution in younger children, under 12 months[17,18], or six months old[11]. It is possible that this difference is related to the small number of children under 12 months of age in this study. Of the 250 children assessed, only 23 (9.2%) were under 1 year of life.

Regarding the physical examination abnormalities at admission, only the changes of the respiratory system were associated with worse prognosis as observed by Sampaio et al.[19] and Alvarenga et al.[20]. Possibly, these changes have already indicated the presence of respiratory infection at admission, since these infections are among the leading causes of death in patients with VL[21].

Among laboratory tests, we found an association between platelets <85,000/mm^3 and unsatisfactory evolution, as described in medical literature[22,23]. The Leishmaniasis Surveillance and Programe of the Brazilian Ministry of Health recommends hospitalization for patients with platelet counts <50.000/mm^3, however, in our series, the association between unsatisfactory evaluation has already occurred with count of 85,000/mm^3, suggesting that if these patients are not hospitalized, at least they should be followed carefully. In the same way, hemoglobin <9.0mg/dl was used as a trial to improve severity criteria sensitivity, once hemoglobin <7.0mg/dl was not associated with unsatisfactory evaluation.

Being from Metropolitan Area of Belo Horizonte was associated with better prognosis of the VL, possibly due to easier access to health care in the capital or surrounding area when compared to the countryside, a fact verified by Pastorino et al[24]. in São Paulo and Campos[15] in Brasília.

Jaundice, liver enlargement, anemia, elevated transaminase levels, reduction in prothrombin time, hypoalbuminemia and elevation of bilirubin were associated with unsatisfatory evolution, as described by other authors, but were not significant in multivariate model[11,12,14,24]. This fact is probably associated to the high percentage of loss of information found on these variables, which impairs proper analysis of the data, especially the performance of logistic regression.

Because it is based on retrospective chart review, lack of information, either by lack of the information itself or lack of systematic record of it, is the main limitation of this study. Despite this limitation, the study showed that in relation to age and platelet count, perhaps the ideal cutoff point to indicate a worse prognosis is slightly higher than the one currently adopted, 18 months instead of 12 months and 85,000 platelets/mm^3 instead of 50,000. Changing the cutoff point of these variables may increase the

sensitivity of severity criteria currently adopted by the Brazilian Ministry of Health, but it may also reduce its specificity, increasing the percentage of false-positive cases (patients without risk severe diseases classified as at risk). On the other hand, the early identification of potential severe cases allows the most appropriate treatment, as the earlier use of amphotericin B deoxycholate, the transfer to units with advanced life support, and the awareness for the most frequent complications, such as respiratory infections.

In summary, the age of < 18 months, the presence of abnormal respiratory physical examination, and thrombocytopenia (< 85,000/mm^3) were statistically associated with poor prognosis of VL, while being from the Metropolitan Area of Belo Horizonte was the only factor with a protective effect in this population. These results suggest that patients aged between 12 and 18 months, with respiratory abnormalities and with platelets counts bellow 85,000/mm^3 should be considered potentially severe, and should be prompt evaluated for respiratory tract infections.

ACKNOWLEDGMENTS

The authors thank Dr. Vitória Mourão for the critical reading and suggestions that improved this paper.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Centro de Pesquisas René Rachou (CPqRR), *Fundação Oswaldo Cruz* and CNPq - *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (FIOCRUZ).

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