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Risk factors associated with death in Brazilian children with severe dengue: a case-control study

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OBJECTIVE: The purpose of this case-control study was to evaluate risk factors associated with death in children with severe dengue.

METHODS: The clinical condition of hospitalized patients with severe dengue who died (cases, n = 18) was compared with that of hospitalized patients with severe dengue who survived (controls, n = 77). The inclusion criteria for this study were age under 13 years; hospital admission in São Luis, northeastern Brazil; and laboratory-confirmed diagnosis of dengue.

RESULTS: Severe bleeding (hemoptysis), a defining criterion for dengue severity, was the factor most strongly associated with death in our study. We also found that epistaxis and persistent vomiting, both included as warning signs in the World Health Organization (WHO) classification of dengue, were strongly associated with death. No significant association was observed between any of the laboratory findings and death.

CONCLUSIONS: The finding that epistaxis and persistent vomiting were also associated with death in children with severe dengue was unexpected and deserves to be explored in future studies. Because intensive care units are often limited in resource-poor settings, any information that can help to distinguish patients with severe dengue with a higher risk to progress to death may be crucial.

KEYWORDS: Dengue; Child; Risk Factors; Death; Brazil; Case-Control Studies.

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INTRODUCTION

The incidence of dengue virus infection is rising in endemic areas in tropical and subtropical regions worldwide. In the Americas, the cumulative number of dengue cases in the last 30 years exceeded 5 million. In this time period, Brazil had the largest number of reported dengue cases (54.5% of those reported in the Americas) and the sixth largest number of dengue hemorrhagic fever (DHF) cases (1). In Brazil, the first dengue outbreak occurred in 1981-1982, in Boa Vista, Roraima State, with isolation of the DENV-1 and DENV-4 serotypes (2). This outbreak was contained by local vector control measures, and no dengue activity was reported for the next 4 years (3). In 1986, the DENV-1 serotype was introduced into Rio de Janeiro. Since the re-emergence of dengue in Brazil in 1986, the country has had several epidemics and has reported the highest number of cases in the world on multiple occasions (4). In the Americas, the dengue case-fatality rate has increased over the past decade (1). The incidence of severe cases of dengue in Brazil has increased since 2001 (3,5), with a dramatic increase in severe cases and dengue-related deaths in patients younger than 15 years of age since 2007, particularly in the northeastern region of the country (6-7).

Three serotypes of dengue virus (DENV-1, DENV-2, and DENV-3) have been endemic in Brazil since 2000 (4,8). Endemic circulation of the fourth serotype (DENV-4) has recently also been confirmed in Brazil (9-10). In the northeastern state of Maranhão, high household infestation rates of *Aedes aegypti* have been observed since 1995, particularly on São Luís Island (11). The first few cases of dengue caused by DENV-1 in Maranhão were reported in

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1994, followed by outbreaks in 1995-96 and 1997-98 (12). A seroepidemiologic survey conducted in 1996 revealed a 41.5% prevalence of dengue antibodies among residents of São Luís Island (13). After the introduction of DENV-2 into Maranhão in 2001, the incidence of dengue in the state increased (12); however, the first deaths from DHF only occurred after the introduction of DENV-3 (5,12). An increase in the incidence of DHF and an increase in case-fatality rates in children under age 15 during a 2006-2007 epidemic were predominantly associated with infection with the DENV-2 serotype. The risk factors for DHF and for dengue shock syndrome (DSS) in children have been addressed in previous studies (14-20). However, most clinical and laboratory findings associated with death in children have been compiled through descriptive studies. In this case-control study, we report the risk factors associated with death in dengue patients younger than 13 years.

MATERIALS AND METHODS

Study design and patients

This study was performed in São Luís, the capital of the northeastern Brazilian state of Maranhão. The inclusion criteria for this study were age under 13 years and hospital admission in São Luis with laboratory-confirmed acute dengue infection. The patients who died (n = 18) were selected among patients admitted to any hospital in São Luís from April 2006 (the beginning of the epidemic) through December 2007. The controls (n = 77) were all patients with severe dengue admitted to the Hospital of the Universidade Federal do Maranhão (HUUFMA) during the same period who survived. The controls were selected only in the HUUFMA because it is a state referral hospital for the treatment of severe and complicated dengue cases where reliable medical records were available. It is a public hospital that is part of the national public health system. Additionally, we conducted a subset analysis, considering only the cases admitted to the HUUFMA.

Laboratory diagnosis of acute dengue infection

The diagnosis of acute dengue infection was confirmed by detection of dengue-specific IgM antibodies using an immunoglobulin M antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) or by DENV detection in serum, blood, or viscera by reverse transcription-polymerase chain reaction (RT-PCR). These tests were conducted in a Central Public Health Reference Laboratory.

Dengue case classification

The 2009 World Health Organization (WHO) ''Dengue guidelines for diagnosis, treatment, prevention, and control'' document was used for dengue case classification and for determining levels of severity. In this classification, severe dengue is defined by the presence of severe plasma leakage leading to shock or fluid accumulation with respiratory distress; and/or severe bleeding, as evaluated by the clinician; and/or severe organ involvement (liver, central nervous system [CNS], heart and other organs) (21).

Clinical and laboratory data

Demographics, medical history, clinical findings, results of laboratory tests and imaging as well as information about treatment and patient outcomes were obtained from medical records and investigation forms of the National Mandatory Reporting System (SINAN) for dengue cases. Patients who died were identified through data available in three different systems: the SINAN, the Mortality Information System (SIM) and the Hospital Admission Information System of the National Public Health System (SIH-SUS). Additional clinical and pathological information regarding fatal cases was obtained from death certificates, necropsy reports and interviews with family members compiled by the Municipal Dengue Control Program of São Luís.

The plasma leakage criteria were cavity effusion (found on imaging or on necropsy), hemoconcentration (an increase in hematocrit of $\geq$20% of baseline or a decrease in hematocrit following volume-replacement treatment of $\geq$20% of baseline), hypoalbuminemia (serum albumin $<$3.5 g/dL) or hypoproteinemia (serum protein $<$6.0 g/dL).

Data analysis

Clinical and laboratory data were entered into a database using Epi Info 3.5 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). STATA 10.0 software (StataCorp LP, College Station, TX, USA) was used for statistical analysis. For quantitative variables, measures of central tendency and dispersion were calculated. Qualitative variables are presented as frequencies and proportions. A logistic regression model was used to calculate the unadjusted associations between the outcome (death) and various independent variables. Odds ratios and 95% confidence intervals were calculated. The number of cases was too small to allow for adjustment in a multiple logistic regression model.

Ethics

The study protocol was approved by the Institutional Review Board of the HUUFMA. Sources included information obtained from medical records, death certificates, necropsy reports and SINAN investigation forms. Confidentiality and subject anonymity was ensured throughout the investigation. Written informed consent was not obtained because the study primarily relied on secondary data. In interviews with the family members of patients who died of dengue, oral consent was obtained and documented.

RESULTS

During the study period, 33 patients under 13 years of age died of suspected dengue. Ten of these 33 patients were admitted to the ICU of the HUUFMA, and 23 were admitted to one of the seven other hospitals in São Luís. Of the 33 patients, 18 had laboratory-confirmed dengue infection and were included in the study as cases. Thirteen of the remaining 15 patients who died could be thoroughly investigated, and 4 had signs of severe plasma leakage, suggesting severe dengue. However, because a laboratory diagnosis of dengue could not be confirmed, these 4 patients were not included in the study. Of the 18 cases included in the study, 5 were admitted to the ICU of the HUUFMA.

Of 396 patients younger than 13 years with suspected dengue who were admitted to the HUUFMA during the study period, 77 had laboratory-confirmed dengue in its most serious presentation (shock) and were included in the study as controls. Demographic information, DENV

Table 1 - Demographic and clinical features of children with severe dengue.

Demographic or clinical feature	Cases	Controls
No. of patients	18	77
Time of occurrence		
2006	3	44
2007	15	33
Boys, no. (%)	6 (33.3)	34 (44.2)
Admission to ICU, n (%)	11 (61.1)	12 (15.6)
Serotype (n)	DENV-2 (5)	(0)
Necropsy	6	0
Comorbidities	1 (5.5)	0
Suspected dengue on admission, n (%)	9/13 (69.2)	76/77 (98.7)
Age, mean years ± SD (median)	4.17±3.29 (4)	4.04±2.72 (4)
Age range in years	0-10	0-12
Age, minimum in months	6	4
Fever duration, mean days ± SD (median)	3.77±1.89 (3)	5.30±2.53 (5)
Duration of fever, range in days	1-8	1-13
Hospital stay, mean days ± SD (median)	2.20±2.01 (2)	7.26±3.63 (6)
Hospital stay, range in days	0-6	3-24

serotypes, ICU admission and the durations of fever and hospital stay are shown in Table 1. All cases and controls had fever. The durations of fever and hospital stay were shorter among cases than controls (Table 1).

Simple logistic regression analysis showed that epistaxis, hemoptysis and persistent vomiting were clinical signs significantly associated with death (Table 2). Table 3 shows the laboratory and imaging results as well as the analysis of the plasma leakage criteria (cavity effusion, hemoconcentration, hypoalbuminemia and hypoproteinemia). No significant association was observed between any of the laboratory findings and death.

To control for possible selection bias, we performed a subset analysis including only the cases admitted to the HUUFMA (Table 4). In this subset, most of the findings were maintained. The association with ''persistent vomiting'' was no longer significant; however, epistaxis remained a significant factor associated with death.

DISCUSSION

In our study, no significant association was observed between any of the laboratory findings and death. This finding supports the 2009 WHO dengue criteria for severe dengue, which emphasize clinical signs over laboratory findings (21). Severe plasma leakage leading to shock did not appear to be a significant risk factor because all controls had severe dengue with shock. The factor most strongly associated with death in our study was severe bleeding (hemoptysis). Hemoptysis is considered a defining criterion for dengue severity, according to the revised WHO classification (21) that was recently assessed in a multicenter study (22). However, we found that epistaxis and persistent vomiting, which are considered warning signs in the revised WHO dengue case classification, were also strongly associated with death. Epistaxis remained significantly associated with death in the subset analysis including only the cases admitted to the HUUFMA. From a clinical point of view, epistaxis and persistent vomiting are not intrinsically severe, unlike hemoptysis and shock, which can cause a rapid progression of the patient to death. However, epistaxis and persistent vomiting can be surrogate markers for severe dengue, even if we do not currently have a logical explanation for this finding.

Signs and symptoms associated with death in children have been addressed in previous descriptive studies. In Thai children, bleeding was one of the risk factors for DSS (17). In Colombian children with DHF and atypical manifestations of dengue, all those who had hemoptysis died (23). In Malaysian children with severe dengue infections, a significant association was found between major bleeding and death ($p = 0.001$) (24). In an Indonesian study of 30 children with dengue who died, 16.7% had epistaxis (25). In the 1981 Cuban epidemic, of the 13 children with DHF/DSS who died, 12 had vomiting, and 3 had epistaxis (26).

In a descriptive study of 15 Colombian children younger than 13 with DHF who died, the cause of death was myocarditis in 9, acute hepatitis in 3 and disseminated intravascular coagulation in the remaining 3, indicating that mortality due to DHF was not caused only by hypovolemic shock (27). Some information from the cited descriptive

Table 2 - Association between clinical characteristics and death in children with severe dengue.

Signs and symptoms	Cases N (%)	Controls N (%)	Odds ratio	95% CI	*p*-value
Cold extremities	8/17 (47.1)	14/77 (18.2)	4.00	1.31-12.19	0.015
Cyanosis	10/17 (58.8)	17/77 (22.1)	5.04	1.67-15.24	0.004
Dehydration	14/15 (93.3)	53/77 (68.8)	6.34	0.79-51.01	0.083
Dyspnea	11/17 (64.7)	36/77 (46.7)	2.09	0.70-6.21	0.186
Edema	8/17 (47.1)	47/77 (61.0)	0.57	0.20-1.63	0.293
Lethargy	13/17 (76.5)	36/77 (46.7)	3.70	1.11-12.37	0.034
Persistent vomiting	7/17 (41.2)	11/77 (14.3)	4.20	1.32-13.37	0.015
Prostration	11/17 (64.7)	9/77 (11.7)	13.85	4.12-46.62	<0.001
Restlessness	6/17 (35.3)	13/77 (16.9)	2.68	0.84-8.56	0.095
Shock	18/18 (100.0)	77/77 (100.0)	NC[a]	NC	NC
Bleeding (any kind)	15/18 (83.3)	77/77 (100.0)	NC	NC	NC
Ecchymoses	6/18 (33.3)	13/77 (16.9)	2.46	0.78-7.75	0.124
Epistaxis	5/17 (29.4)	7/77 (9.1)	4.17	1.13-15.3	0.032
Gastrointestinal bleeding	14/18 (77.8)	41/77 (53.3)	3.07	0.93-10.18	0.066
Gum bleeding	1/17 (5.9)	10/77 (13.0)	0.42	0.05-3.51	0.422
Hemoptysis	4/17 (23.5)	1/77 (1.3)	23.38	2.42-226.1	0.006
Petechiae	6/18 (33.3)	54/77 (70.1)	0.21	0.07-0.64	0.006
Positive tourniquet test	1/3 (33.3)	15/31 (48.4)	0.53	0.04-6.51	0.622

[a]NC = not calculated.

Table 3 - Laboratory and imaging findings of children with severe dengue.

Variable	Cases N (%)	Controls N (%)	Odds ratio	95% CI	*p*-value
ALT ≥1000 U/L	1/12 (8.3)	1/70 (1.4)	6.27	0.36-107.78	0.206
AST ≥1000 U/L	2/12 (16.7)	3/73 (4.1)	4.67	0.69-31.45	0.114
Hematocrit >45%	3/12 (25.0)	21/77 (27.3)	0.89	0.22-3.60	0.869
Leukocyte count >10 000/mm^3	5/12 (41.7)	13/77 (16.9)	3.52	0.96-12.82	0.057
Platelet count					
<150 000/mm^3	11/14 (78.6)	73/77 (94.8)	0.20	0.04-1.02	0.053
<100 000/mm^3	11/14 (78.6)	61/77 (79.2)	0.96	0.24-3.86	0.956
<50 000/mm^3	6/14 (42.9)	35/77 (45.5)	0.90	0.28-2.84	0.857
Findings on imaging exams					
Ascites	7/8 (87.5)	36/54 (66.7)	3.50	0.40-30.66	0.258
Pleural effusion					
on ultrasonography	5/8 (62.5)	39/54 (72.2)	0.64	0.14-3.02	0.574
on radiography	5/9 (55.6)	56/71 (78.9)	0.33	0.08-1.40	0.135
Thicker gallbladder wall	3/8 (37.5)	29/54 (53.7)	0.52	0.11-2.38	0.398
Plasma leakage criteria					
Cavity effusion	17/18 (94.4)	69/74 (93.2)	1.23	0.13-11.25	0.853
Hemoconcentration	5/7 (71.4)	49/76 (64.5)	1.38	0.25-7.58	0.713
Serum albumin <3.5 g/dL	7/7 (100.0)	64/70 (91.4)	NC[a]	NC	NC
Serum protein <6.0 g/dL	5/5 (100.0)	43/51 (84.3)	NC	NC	NC

[a]NC = not calculated.

studies on death in children with dengue is summarized in Table 5.

The DENV-2 serotype predominated in Maranhão in 2006 and 2007. Unfortunately, genotyping was not performed for all patients in the outbreak in São Luís; the DENV serotype was determined in only 5 cases, and all 5 were DENV-2.

In our study, different factors may have contributed to the poor prognosis of the cases. The pediatricians had no experience in the diagnosis and management of patients with dengue, and health teams were not prepared to provide emergency care to patients with severe dengue during the early phase of the epidemic. Only 69.2% of cases were diagnosed as dengue at the time of hospital admission, compared to 98.7% of controls. This finding suggests that a delay in dengue diagnosis may have worsened the prognosis. Additionally, 5 patients had no access to an ICU bed, and 2 arrived at the hospital with advanced disease. Similar findings were observed in a descriptive study on 14 deaths due to dengue in 2 municipalities in northeastern Brazil (30). The durations of fever and hospital stay were shorter in cases than in controls, suggesting that the clinical condition of the cases on admission was worse than that of the controls, which may have contributed to the worse outcomes observed for the cases. It is important to consider that the earlier a risk factor for death can be identified, the greater the possibility of introducing appropriate therapeutic interventions to prevent death. If high-risk factors had been promptly recognized and the children had been properly treated, it is possible that progression to profound shock and death could have been prevented.

The selection of the cases at 7 different hospitals, which could have health care resources of varying quality, may have contributed to the deaths of the case patients.

The small number of cases in this study resulted in very wide confidence intervals, including the variables strongly associated (odds ratio≥3) with death. This limitation also prevented us from adjusting the data in a multivariate logistic regression model. Our unadjusted analysis showed

Table 4 - Association between clinical characteristics and death in children admitted to the Hospital of the Universidade Federal do Maranhão with severe dengue.

Signs and symptoms	Cases N (%)	Controls N (%)	Odds ratio	95% CI	*p*-value
Cold extremities	3/5 (60.0)	14/77 (18.2)	6.75	1.03-44.26	0.047
Cyanosis	5/5 (100.0)	17/77 (22.1)	NC[a]	NC	NC
Dehydration	4/5 (80.0)	53/77 (68.8)	1.81	0.19-17.08	0.604
Dyspnea	5/5 (100.0)	36/77 (46.7)	NC	NC	NC
Edema	5/5 (100.0)	47/77 (61.0)	NC	NC	NC
Lethargy	4/5 (80.0)	36/77 (46.7)	4.56	0.49-42.64	0.184
Persistent vomiting	0/5 (0.0)	11/77 (14.3)	NC	NC	NC
Prostration	3/5 (60.0)	9/77 (11.7)	11.33	1.66-77.27	0.013
Restlessness	2/5 (40.0)	13/77 (16.9)	3.28	0.50-21.64	0.217
Shock	5/5 (100.0)	77/77 (100.0)	NC	NC	NC
Bleeding (any kind)	5/5 (100.0)	77/77 (100.0)	NC	NC	NC
Ecchymoses	2/5 (40.0)	13/77 (16.9)	3.28	0.50-21.24	0.217
Epistaxis	4/5 (80.0)	7/77 (9.1)	40.0	3.91-409.05	0.002
Gastrointestinal bleeding	5/5 (100.0)	41/77 (53.3)	NC	NC	NC
Gum bleeding	1/5 (20.0)	10/77 (13.0)	1.67	0.17-16.54	0.659
Hemoptysis	2/5 (40.0)	1/77 (1.3)	50.67	3.53-726.74	0.004
Petechiae	4/5 (80.0)	54/77 (70.1)	1.70	0.18-16.08	0.642
Positive tourniquet test	1/2 (50.0)	15/31 (48.4)	1.07	0.06-18.62	0.965

[a]NC = not calculated.

Table 5 - Summary of descriptive studies on dengue deaths in children.

Author(s), year	Forms of disease	Age	Number of patients	Number of deaths	Symptoms associated with death or cause of death, n (%)
Méndez, González, 2006 (23)	DHF/DSS	0 m-12 y	168	10	Hepatitis 6/10 (60.0), Neurological alterations 6/10 (60.0)
Sumarmo et al., 1983 (25)	DF/DHF/DSS	0 m-14 y	30	30	GI bleeding 24/30 (80.0), Hepatomegaly 16/30 (53.3), Petechiae 16/30 (53.3)
Guzmán et al., 1984 (26)	DHF/DSS	2 m-12 y	13	13	Vomiting 12/13 (92.3), Hematemesis 12/13 (92.3), Ascites 9/13 (69.2)
Salgado et al., 2008 (27)	DHF/DSS	0 y-12 y	13	13	Myocarditis 9/13 (69.2), Hepatitis 3/13 (23.1), DIC 3/13 (23.1)
Kamath, Ranjit, 2006 (28)	DHF/DSS	Children	109	9	Shock/DIC/ARDS 4/9 (44.4), Shock/DIC 2/9 (22.2), Neurological alterations 2/9 (22.2)
Kouri et al., 1989 (29)	DHF/DSS	Children	124	57	GI bleeding

that severe bleeding manifested by hemoptysis was strongly associated with death, providing additional support for the revised WHO dengue case classification (21). However, the finding that epistaxis and persistent vomiting were also associated with death in children with severe dengue was unexpected and deserves to be explored in future studies. Because intensive care units are often limited in resource-poor settings, any information that can help to distinguish patients with severe dengue with a higher risk to progress to death may be crucial.

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AUTHOR CONTRIBUTIONS

Branco MR, Luna EJ and Pannuti CS conceived and designed the study, performed the final data analysis and prepared the first draft. Branco MR, Braga Jr LL, Oliveira RV, Rios LT, Silva MS, Medeiros MN, Silva GF, Nina FC, Lima TJ, Brito JA and Oliveira AC collected the data. All authors contributed to the revision of the manuscript and read and approved its final version.

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