

Original Research Article

Factors associated with anemia in children with *P. vivax* malaria from Brazilian Amazon basin

Abstract

Mild anemia is common in children with vivax malaria, but several factors may lead to severe cases. Thus, elucidates these factors in different endemic areas is relevant to prevent an unfavorable prognosis of the disease. The aim of the present study was to investigate the incidence and evaluate contributing factors to anemia in children with vivax malaria infection that live in a municipality in the west of the Brazilian Amazon basin. Data were collected during clinical interviews with the guardians of children and hemoglobin was measured by spectrophotometry. All data were collected at admission in the study. A total of 48 children were enrolled in the study. The mean age was 8 years and the distribution of genders was similar among participants. At admission, all children were symptomatics and the guardians reported previous episodes of malaria. A total of 48 children were enrolled in the study. The main signals and symptoms were chills, headache, and gastrointestinal disorders. Anemia occurred in 56 % of children. Hemoglobin levels were not correlated with parasitemia in anemic or non-anemic children. The variation of hemoglobin levels at admission was explained by both the gender and the fever at admission, which should be considered as potential determining factors for anemia in children.

Key-Words: Malaria; Anemia; Plasmodium vivax; Hemoglobin.

1. Introduction

Malaria remains an important public health problem worldwide, with approximately 3 billion people at risk of contracting the disease [1]. In Brazil, approximately 143,000 cases occurred

in 2015, most of them in the Amazon basin. *Plasmodium vivax* was the most prevalent species and accounted for 85 % of the cases. In this endemic area, the transmission is unstable and heterogeneous as well as occurs in all age groups and in both genders [2, 3].

The infection may be life threatening for pregnant women and children. Anemia is **ones** of the most frequent causes of severity. Mild anemia is common in children with vivax malaria, but it may become severe after relapsing infections, in children with malnutrition or with concurrent infections such as dengue fever and HIV [4-6]. The main causes are the destruction of parasitized and unparasitized erythrocytes in the peripheral circulation, spleen and bone marrow, the dyserythropoiesis and the use of anti-malarial drugs [6-9]. Moreover, several factors increase the risk of anemia in these patients, such as gender, history of the disease, parasitemia at admission and Plasmodium species [10-12].

In the South America, only a few studies focused on contributing factors for anemia in children with vivax malaria [10, 12]. Therefore, the aim of the present study was to investigate the occurrence of anemia in children with vivax malaria from a municipality with high transmission rate in the western region of the Brazilian Amazon basin. Furthermore, we sought to elucidate whether gender, age, fever, and parasitemia at admission, history of illness and number of previous episodes of the disease contributes to the variation of the hemoglobin levels at admission to the treatment.

2. Material and methods

2.1. Study site and participants

The study is a part of a major research project designated to evaluate the health conditions of inhabitants of the Marajo Island. The municipality of Anajas (00° 59' 21"S and 49° 56' 24"W) was chosen to carry out the study **because its notify 17.4 % of malaria cases** in the state of Para and has an annual incidence of cases above 50/1.000 inhabitants. Most cases

(86.3%) are caused by *P. vivax*. The municipality has an area of 6.913 Km² and 28.012 inhabitants, of which 7.347 are children aged 2-10. In 2014, the disease occurred in approximately 36.6 % of these children. The human development index is very low (0.484), and the economy is based on agriculture practices, timber extraction, and fishing. The dry season is from June to November and the annual rainfall is 1.800- 3.050 mm. Temperatures range from 22°C to 32°C. The annual relative humidity is 80-90 %.

Participants were randomly recruited among those who spontaneous searched for attendance at health facilities of the municipality from January to December 2014 with signals and symptoms suggestive of malaria, with posteriorly confirmed blood smears as positive infection by *P.vivax*. Exclusion criteria included patients with mixed malaria or signs and symptoms of severe malaria (jaundice, renal or pulmonary impairment, severe anemia, altered level of consciousness), G6PD deficiency, chronic and other parasitic diseases mainly gastrointestinal helminth, obesity and underweight and who has reported a history of malaria infection within the previous three months.

Children were treated with chloroquine and primaquine according to the recommendation of the World Health Organization [4]. The community health workers were responsible for prescribing and dispensing the drugs under the supervision of the medical staff. The guardians of children received specific instructions about the administration on each day and were strongly advised to complete the course of the treatment.

2.2. Data collection

A clinical questionnaire was applied to all guardians of children at their enrollment in the study. The data recorded from each child were age, gender, fever (presence or absence) of axillary temperature above 37.5°C, previous episodes of the disease (number of episodes) and the history of illness before admission.

74 **2.3. Hemoglobin estimates**

75 Blood was collected at admission using Vacutainer system, containing EDTA-K3 (Becton-
76 Dickinson) to a total volume of 4.5 ml. The measure of hemoglobin was based on
77 photometric detection of **cyanmethemoglobin** that is a stable compound derived from
78 hemoglobin, using Merck- diagnosis Kits, following the good laboratory practices. The
79 diagnosis of anemia and its severity followed the criteria of the World Health Organization
80 [13].

81 **2.4. Parasite count**

82 Parasite counts were performed in thick blood smears prepared as recommended by the
83 Walker technique and evaluated by an experienced microscopist using 100X (oil immersion)
84 objectives. Parasite density was expressed as the number of parasites per microliter of blood,
85 which was derived from the number of parasites per 200 white blood cells in the thick film,
86 considering a total white blood cell count of 8.000. The limit of detection of parasites was
87 40/ μ L [14].

88 **2.5 Data analysis**

89 The data are described as mean, geometric mean and standard deviation. Chi-squared test (χ^2)
90 was used to compare qualitative variables between groups of the study. The quantitative
91 variables were compared **betweeen** anemic and non-anemic children by the test *t* of student, if
92 normally distributed or alternatively, by the Mann-Whitney U test. Correlation between
93 parasitemia and hemoglobin levels at admission was estimated by the coefficient of
94 correlation of Spearman. A generalized linear mixed model was used to confirm the factors
95 accounts for the variation of hemoglobin levels. The variables explanatory chosen were
96 gender, age, fever at admission (presence or absence of axillary temperature above 37.5 0C),
97 parasitemia at admission (below or above 10.000 mm³), history of illness before admission
98 (interval between the first signals and symptoms and the attendance at health facility,

classified as below or above 48hours) and the history of malaria (classified as below or above 3 episodes of disease). Model selection was based on stepwise backward elimination of the non-significant terms in the order of their significance assessed by their Wald statistic. All eliminated terms were reintroduced to the final model to confirm their lack of contribution and to check how they affected the fit of the model. We used the normal error and the identity link function for modeling. All p-values were two-tailed, and $p < 0.05$ was considered significant. Statistical analyses were performed with STATISTICA software package (Version 7.0, Stat Soft Inc. 2004, Tulsa, USA).

2.6 Ethical statement

The study is a part of a major research project submitted to Plataforma Brasil under protocol CAAE 2 07199612.0.0000.0018 and approved under the number 261.593/2013.

3. Results

A total of 48 children met the criteria for inclusion in the study. Considering all patients, the mean age of all children was 8 years (range from 6-10 years). The distribution of genders was similar among participants of the study ($\chi^2=1.3$; $p=0.403$). All children had a history of malaria, with a median of 4 (range from 2-5) earlier episodes of the disease. The geometric average of parasitemia at admission was 1365 (4.8) mm^3 . The most prominent clinical findings at admission were: headache (95 %), chills (43 %), vomiting (44 %), diarrhea (31 %), anorexia (41 %) asthenia (35 %) and fever (35 %). The average time elapsed between first signals and symptoms and the health attendance was 71 hours (range from 24-144 hours). Anemia occurred in 56 % of children ($n=27$) and was moderate in most cases. Then the children were allocated into two groups, anemics and non-anemics. The baseline characteristics of children in each group are in Table 1.

123 Table 1. Baseline characteristics of children. Data are expressed as mean and standard
124 deviation.

Characteristic	anemic (n=27)	non-anemic (n=21)	p-value
Gender, (%)			
male	40	85	0.008*
female	60	15	
Age, years	8 (1)	8(1)	0.272**
Parasitemia at admission, geometric average	1123 (4.0)	2015 (4.13)	0.131**
Previous episodes of disease, median	4.5 (2)	4.0(2)	0.726***
History of illness, hours	64(28)	75(29)	0.296 **
Fever at admission, %	50	16	<0.0001*
Fever clearance, hours	24(12)	26(8)	0.590**
Parasite clearance, hours	36(12)	36(12)	0.99**
Hemoglobin, g/l	98(10)	125(7)	<0.0001**
Hematocrit, %	27.8 (2.7)	32.8(2.8)	<0.0001**
Red blood cells, millions	3.44 (0.5)	4.28(0.49)	<0.0001**

125

126 *chi-squared test; ** student t-test; *** Mann-Whitney U test.

127 At admission, there were no significant correlations between hemoglobin levels and
128 parasitemia in anemic (rs= -0.086; p= 0.721) or non-anemic (rs= 0.271; p=0.344) children.
129 Analysis of the full model showed that the variation in hemoglobin levels was explained by
130 the gender of children (Wald statistics=22.74; p<0.0001) and by the fever at admission (Wald
131 statistics=17.13; p<0.001) (Figure 1). The other variables were not associated with the
132 variations of the hemoglobin levels: parasitemia at admission (Wald statistics= 1.32;
133 p=0.312), history of malaria (Wald statistics= 0.521; p=0.500) and the history of illness
134 (Wald statistic= 0.312; p=0.514).

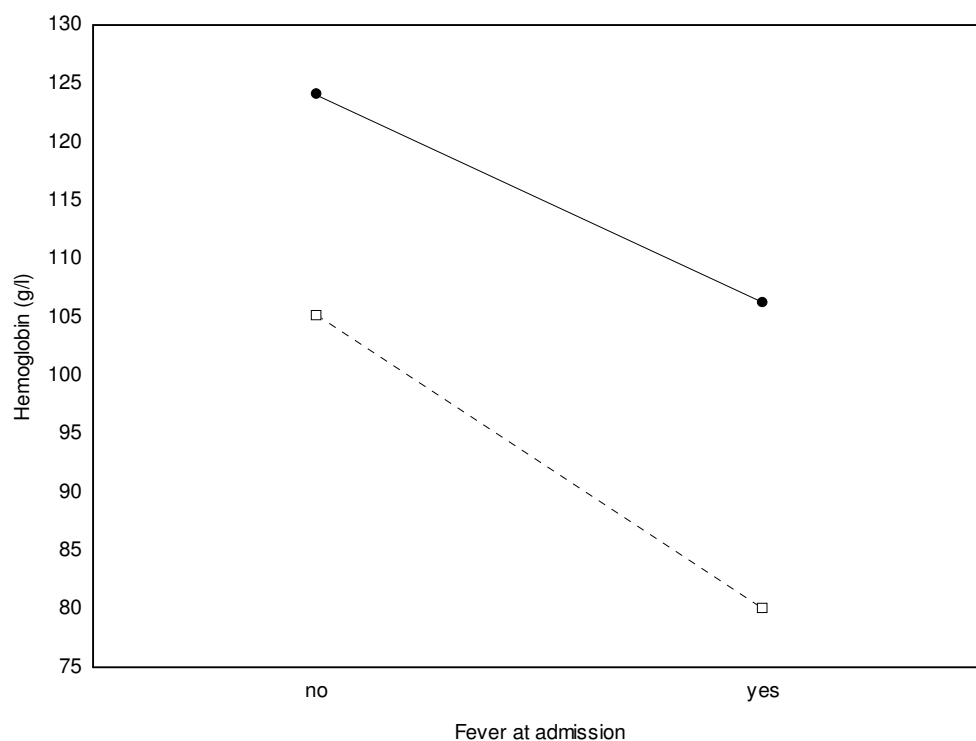


Figure 1: Hemoglobin levels (g/l) of male (●-●) and female (□-□) children according the occurrence of fever at admission.

3. Discussion

Malaria is one of the most common causes of morbidity and mortality in children worldwide. The severe anemia and the cerebral impairment are responsible for most of the malaria-related deaths [5, 6, 7, 15, 16, 17]. The data revealed a similar distribution of the disease in both gender and the occurrence of previous episodes of the disease in all children enrolled in the study, which is in line with previous studies done in the Brazilian Amazon basin [3, 7, 11, 12].

The clinical manifestations of malaria, the severity, and course of a clinical attack depends on the species of the infecting Plasmodium and on human host related factors as age, ethnicity, immune and nutritional status. At admission, the children enrolled in the study showed low

149 parasite density and were symptomatic with the prevalence of mild signals and symptoms
150 such as chills, headache, and gastrointestinal impairment. These findings are common in
151 children living in areas of low to medium transmission rates and with a history of several
152 episodes of malaria. The recurrent episodes confer partial immunity to the human host,
153 including children, contributing to the appearance of the asymptomatic cases that represent a
154 challenge for the *P. vivax* elimination [4, 5, 6, 18, 19].

155 Mild to moderate anemia was found in 58% of children enrolled in the study. These results
156 agreed with several cross-sectional studies carried out in the Brazilian Amazon basin that
157 found frequencies of anemia as high as 80% in children with malaria [7, 11, 12, 17, 20, 21].
158 The gender and the fever at admission explained the variation of the hemoglobin levels.
159 Female children showed lower hemoglobin levels compared to male children. Although the
160 data of the study have suggested a higher risk of anemia in female children than in male
161 children, it should be considered that female patients are a risk group, regardless of malaria
162 since they have a lower threshold for anemia following a hematological insult [20, 22, 23,
163 24].

164 The fever is a key symptom of malaria and ones of the most frequent cause of search for
165 health attendance. At admission, febrile children corresponded to 35% of those enrolled in the
166 study. Moreover, the fever was responsible for the variation in hemoglobin levels with the
167 lowest values in febrile patients. The small number of febrile children at admission could be
168 explained by the asynchronous life cycle of the parasites at initial phase of infection, the
169 partial degree of immunity of these children that could requires a higher degree of parasite
170 density for onset of symptoms and the interindividual variations regarding to the degree of
171 parasitemia required to induce the symptoms. These factors could be responsible by the
172 nonappearance of the classical cycle of *P. vivax* infections every 48 hours in most cases of
173 infection [5, 6, 25, 26].

Significant associations between anemia and prolonged history of illness were found for the most prevalent species of Plasmodium, however, this association was not found in the study [20, 22]. A plausible explanation is a short time elapsed between the onset of symptoms and diagnosis of the disease due to both to the previous history of the disease as to the easy and free access to diagnosis and treatment in this endemic area. Moreover, there was no significant correlation between hemoglobin levels and parasite density at admission, which suggests a more complex interaction between severity of anemia and intensity of infection [20, 22, 23]. Finally, other potential causes of anemia in population groups living in endemic areas of the Brazilian Amazon basin should be considered such as the low economic status, the micronutrients deficiency, the precarious health conditions and the high occurrence of intestinal parasites [20, 23]. The later was an exclusion criterion of the study, but the analysis of micronutrients was not performed, which can be considered the main limitation of the study.

4. Conclusions

The findings of the present study are clinically relevant as they show the importance of the gender and the fever at admission as potential risk factors for the occurrence of anemia in children with vivax malaria that live in an unstable and heterogeneous area of transmission.

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