Original Research Article

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

Abstract

Anemia is a common and frequently severe consequence of *P. vivax* infection. The incidence rate and the severity depend on several factors such as age, endemic area, and pregnancy. In Brazilian Amazon basin, only a few studies focused on the factors associated with anemia in children with malaria. Thus, the aim of the present study was to investigate the incidence and the contributing factors for anemia in children with vivax malaria infection. The data were collected during the clinical interviews with the guardians of the children and the hemoglobin levels were measured by spectrophotometric method. The number of children included in the study was 48. The average age was 8 years (range from 6-10 years). The distribution of gender was similar among all the children included in the study, but there was a high occurrence of anemia in female children. At admission, all children reported earlier episodes of malaria as well as had shown signs and symptoms of the disease mainly chills, headache, and gastrointestinal disorders. Anemia was found in 56 % of the children included in the study. Hemoglobin levels were not correlated with the parasitemia in both anemic and non-anemic children. However, hemoglobin levels were associated with both the gender and the presence of fever at admission. The latter may be considered risk factors for anemia in this age group.

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 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 one of the most frequent causes of severity [4, 5]. The main causes of anemia 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 conditions contribute to increased incidence rates of anemia in children with vivax malaria, such as gender, history of the disease, parasitemia at admission, Plasmodium species, nutritional status and concurrent infections as dengue fever and HIV [4, 5, 6, 10, 11,12].

Comment [NPR1]: [4-6,10-12]

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 levels of hemoglobin in children with vivax malaria from a municipality in the East region of the Brazilian Amazon basin. Furthermore, we sought to elucidate whether the gender, age, fever at admission, parasitemia at admission, history of illness and number of previous episodes of the disease are associated with the occurrence of anemia at admission to the treatment.

2. Material and methods

2.1. Study site and participants

The study is a part of a research project designed to assess the health conditions of the inhabitants of the Marajo Island. The study was carried out at Anajas (00º 59’ 21”S and 49º

56’ 24”W) in the state of Para. The municipality has an annual incidence index of malaria above 50/1.000 inhabitants. Most cases (86.3%) are caused by *P. vivax*. The municipality has an area of 6.913 Km ^{2} and 28.012 inhabitants, of which 7.347 are children aged 2-10. In 2014, the disease occurred in approximately 36.6 % of the children from the minicipality. 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 millimeters. The annual temperature ranges from 22°C to 32°C. The annual relative humidity is about 80 - 90 %.

Participants were randomly recruited among those who searched for attendance at health facilities of the municipality from January to December 2014 with signs and symptoms suggestive of malaria. The inclusion criteria were children aged 2-12 years with slide-confirmed mono-infection by *P.vivax* and with normal body mass index. Exclusion criteria included those patients with mixed malaria or with signs and symptoms of severe malaria (jaundice, renal or pulmonary impairment, altered level of consciousness), G6PD deficiency, chronic and other parasitic diseases mainly gastrointestinal helminth and who have 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^{0}C, previous episodes of the disease (number of episodes) and the history of illness before admission (interval between the first signs and symptoms and the health attendance).

2.3. Diagnosis of anemia

The hemoglobin concentrations were used for the diagnosis of anemia and assessment of severity. The criteria adopted by World Health Organization for children aged 5 – 11 years at sea level are; non-anemia (above 110 g/l), mild anemia (110- 114 g/l), moderate anemia (80-109 g/l) and severe anemia (lower than 80 g/l). A blood sample was collected from each children using Vacutainer system, containing EDTA-K3 (Becton-Dickinson) to a total volume of 4.5 ml. Hemoglobin measurement was based on the photometric detection of cyanomethemoglobin that is a stable compound derived from hemoglobin, using Merck-diagnosis Kits ™, following the good laboratory practices [13].

2.4. Parasite count

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

2.5 Data analysis

The data are described as mean, geometric mean and standard deviation. Chi-squared test ($\chi 2$) was used to compare qualitative variables. The quantitative variables were compared between anemic and non-anaemic children by the test *t* of student, if normally distributed or alternatively, by the Mann-Whitney U test. The correlation between the parasitemia and the levels of hemoglobin was estimated by the coefficient of correlation of Spearman. 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 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 the inclusion in the study. The average age of children was 8 years (range from 6-10 years). The distribution of gender was similar in 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 parasites was 1365 (4.8) mm^3. The most prominent clinical findings were headache (95 %), chills (43 %), vomiting (44 %), diarrhea (31 %), anorexia (41 %) asthenia (35 %) and fever (35 %). The average time before to 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. The comparison of several variables revealed that both the fever at admission and the distribution of gender were significantly different between anemic and non-anemic patients (Table 1 and Figure 1). Moreover, there were no significant correlations between hemoglobin levels and parasitemia in anemic ($rs= -0.086$; $p= 0.721$) and non-anemic ($rs= 0.271$; $p=0.344$) children.

Table 1. Baseline characteristics of anemic and non-anemic children.

Characteristic	anemic (n=27)	non-anemic (n=21)	p-value
Gender, (%)			
male	40	85	0.008*
female	60	15	

Age, years	8 (2)	8 (2)	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, million	3.44 (0.5)	4.28(0.49)	<0.0001**

Data are expressed as a mean and standard deviation.

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

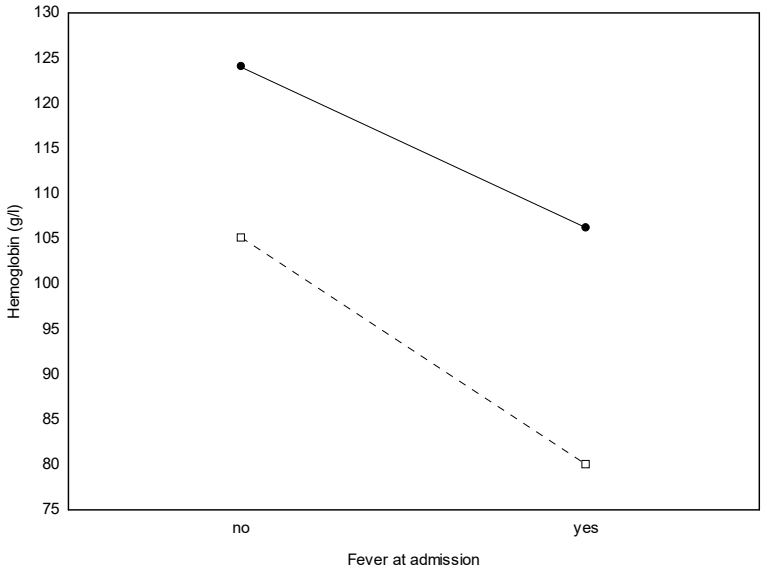

Figure 1: Hemoglobin levels (g/l) of male (●-●) and female (□--□) children according to the presence of fever at admission.

3. Discussion

Malaria is one of the most common causes of morbidity and mortality in children worldwide. Severe anemia and the cerebral impairment are responsible for most of the malaria-related deaths [5, 6, 7, 15, 16, 17]. Thus, clarify the factors that contribute to the anemia is relevant to prevent severe cases of the disease. The data revealed a similar distribution of the disease between genders. Moreover, all children enrolled in the study reported earlier episodes of the disease. These findings are in line with previous studies from the Brazilian Amazon basin [3, 7, 11, 12].

Comment [NPR2]: [5-7, 15-17

The children showed low parasite density, were symptomatic with mild signs and symptoms and with the prevalence of chills, headache, and gastrointestinal impairment. This clinical

134 picture is common in children from endemic areas of low to medium transmission rates with
135 a history of several episodes of malaria. Furthermore, the recurrence of episodes leads to the
136 partial immunity, which contributes to the increased of asymptomatic cases, which are a
137 challenge for the elimination of the disease [4, 5, 6, 18, 19].

Comment [NPR3]: [4-6, 18,19]

138 Mild to moderate anemia occurred in 58% of the children enrolled in the study. This finding
139 agreed with several cross-sectional studies carried out in the Brazilian Amazon basin, which
140 reported frequencies of anemia as high as 80% in children with malaria [7, 11, 12, 17, 20,
141 21].

142 In the study, there was a different distribution of gender between anemic and non-anemic
143 children. Female children showed lower hemoglobin levels compared to male children,
144 suggesting a high risk of anemia in female children. However, only post-pubescent and pre-
145 menopausal women, regardless of malaria, have a lower threshold for anemia following a
146 hematological insult. Moreover, the levels of hemoglobin are similar in pre-puberty female
147 and in male children. Thus, further studies are required to evaluate if there is a significant
148 sex-related difference in the incidence of anemia in children with uncomplicated vivax
149 malaria as reported in hospitalized children or the finding was only a bias due to the number
150 of children included in the study [20, 22, 23, 24].

Comment [NPR4]: [20,22-24]

151 The presence of fever at admission was also different between groups. The fever is a key
152 symptom of malaria and ones of the most frequent cause of search for health attendance. At
153 admission, febrile children corresponded to 35% of those enrolled in the study. Moreover, the
154 occurrence of fever at admission was higher in anemic children compared to non-anemic
155 ones. The small number of febrile children at admission could be explained by several
156 reasons: (a) the asynchronous life cycle of the parasite at initial phase of infection; (b) the
157 partial degree of immunity of children that could requires a higher parasite density for the

onset of symptoms and (c) the interindividual variations related to the parasite density required to induce the symptoms. These factors could be responsible by the nonappearance in most cases of the classical cycle of *P. vivax* infections every 48 hours [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 the short interval between the onset of the signs and symptoms and the health attendance due 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 in both groups, which suggests a more complex interaction between the severity of anemia and the intensity of infection [20, 22, 23].

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, micronutrients deficiency, precarious health conditions and the high occurrence of intestinal parasites [20, 23]. The later was an exclusion criterion of the study. Moreover, anemic and non-anemic children live in similar economic status and health conditions. Finally, the main limitations of the study were the small sample size and the lack of micronutrients analysis.

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 uncomplicated vivax malaria.

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