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ORIGINAL ARTICLE

PREVALENCE OF ANEMIA IN NON-SEVERE MALARIA CASES IN MÂNCIO LIMA, ACRE

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ABSTRACT

Malaria, a parasitic disease, is a serious public health problem. In Brazil, the majority of cases are found in the Amazon. The clinical manifestations of malaria depend on several factors and they may be related to the development of anemia. This study evaluated the prevalence of anemia in malaria cases and its associated factors. The study was conducted in Mâncio Lima (Acre, Brazil). Participants were chosen through passive detection of malaria cases in the municipal health services. They were interviewed and blood samples analyzed for *Plasmodium* detection and for hemoglobin measurement. SPSS 13.0, software was applied for statistical analysis. One hundred and twenty patients with malaria were studied, of which 58.3% male and 41.7% female. There was a 25% prevalence of anemia, mainly among the women, and in those presenting symptoms for more than four days as well as headaches. The prevalence of anemia and its associated factors in these malaria patients may be connected, not only to the infection, but also to previous or overlapping diseases.

KEY WORDS: Anemia; malaria; Mâncio Lima.

RESUMO

Prevalência de anemia em casos de malária não complicada em Mâncio Lima, Acre, Brasil

A malária é uma doença parasitária que constitui um grave problema de saúde pública. No Brasil, os casos estão concentrados principalmente na Amazônia Legal. As manifestações clínicas da malária dependem de vários fatores e a doença está relacionada com o desenvolvimento de anemia. Neste estudo, foi avaliada a prevalência de anemia em casos de malária e fatores associados. O estudo foi realizado em Mâncio Lima, no Acre. Os participantes foram escolhidos

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com base na detecção dos casos de malária pelos postos de saúde do município. Foi aplicado um questionário e amostras de sangue foram analisadas para a pesquisa de plasmódios e para a quantificação de hemoglobina. Valeu-se do SPSS 13.0, *software* para análise estatística. O estudo identificou 120 pessoas com o diagnóstico de malária, sendo 58,3% do sexo masculino e 41,7% do sexo feminino. A prevalência de anemia foi de 25%, associada com sexo feminino, tempo de sintomas superior a quatro dias e presença de cefaleia. A prevalência de anemia e os fatores associados a esses pacientes com diagnóstico de malária podem estar relacionados não somente à infecção, mas também à presença de doenças prévias ou sobrepostas.

DESCRITORES: Anemia; malária; Mâncio Lima.

INTRODUCTION

Malaria is a parasitic disease which, due to its high incidence, is a major public health problem. In 2014 the World Health Organization (WHO) estimated that 198 million cases of malaria had occurred worldwide in 2013, and that the disease accounted for 584,000 deaths in that year (WHO, 2014). The disease is prevalent in tropical regions where favorable socio-economic conditions influence the transmission of the parasite through vector proliferation. (Costa et al., 2010).

In Brazil, the cases are mainly to be found in the Amazon, accounting for 99.9% of the transmissions (Marcondes et al., 2010). In Acre, the incidence of malaria has increased, particularly since the nineteenth century, due to the arrival of northeastern immigrants, migration from rural to urban areas with resulting disorganized population distribution in the towns. (Costa et al., 2010).

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium* and the transmission mechanism is through the bite of the Anopheles mosquito. In Brazil, *Plasmodium vivax* is responsible for most cases of malaria (approximately 90%) while *P. falciparum* is usually responsible for the more severe forms of the disease (Ministério da Saúde, 2010).

The clinical manifestations of malaria depend on several factors, both intrinsic to the host, such as immunity and nutritional status, and factors related to the parasite, such as the species and its relationship with the host (Quintero et al., 2011). *Plasmodium* infection may lead to the development of anemia, defined as low hemoglobin concentration (WHO, 2007).

Malaria is related to the development of anemia in the host by means of a number of mechanisms that have been recently discovered. The first mechanism is the destruction of the cells in the peripheral circulation of the parasite cycle (Quintero et al., 2011). The second mechanism is through cytokine-induced alterations in the bone marrow production of blood cells caused by the immune response of the host (Quintero et al., 2011) and the third mechanism is the presence of auto- antibodies having affinity not only with the parasite, but also with red cells (WHO, 2004; Quintero et al., 2011).

With regard to malaria, most studies suggest a direct relationship

between anemia and high parasitaemia (Noronha et al., 2000; Oliveira et al., 2004); *Plasmodium* species (Douglas et al., 2012; Ronald et al., 2006; Souza-Figueiredo et al., 2012); number of malaria episodes (Douglas et al., 2013); delay in diagnosis (Ventura et al., 1999) and host nutritional state (Ronald et al., 2006). Ronald et al. (2006) reported a 66.2% prevalence of anemia of which 34.5% in children from two communities in Ghana, linked to infection with *P. falciparum*, among other causes.

In Brazil, there are few studies investigating anemia in malaria cases. Ventura et al. (1999) reported anemia in 82.6% of children and adolescents with malaria in Pará State, in the Western Amazon. Cardoso et al. (1992) evaluated the prevalence of anemia among 1,068 inhabitants of a malaria endemic area in Porto Velho, in the eastern Amazon, and reported a prevalence of 44.5% of anemia in malaria patients.

This study evaluated the prevalence of anemia in malaria cases and factors associated with this clinical presentation in an endemic area of malaria in the Brazilian Amazon.

MATERIAL AND METHODS

Study area and population

This study was conducted in Mâncio Lima, Acre, in the western Brazilian Amazon. Mâncio Lima measures 5,453 km^2 and numbers 16,795 inhabitants living in urban (57.3%), rural or riparian (37.9%) and indigenous (4.8%) areas (IBGE, 2010). It is located 38 km from Cruzeiro do Sul and 650 km northwest of the state capital, Rio Branco. It borders the municipality of Cruzeiro do Sul and Rodrigues Alves to the east, Amazonas state to the north and Peru to the west. Mâncio Lima is an equatorial region of palm groves and rainforests (Acre, 2010). The monsoon is from November to April and there is an annual rainfall of 1,600–2,750 mm. Temperatures ranges from 20°C to 32°C. The annual relative humidity is 80–90%. In 2010, the human development index was 0.625. The main sources of income are cattle raising, fishing as well as producing and selling banana and cassava products. In 2013, Mâncio Lima registered 6,936 cases of malaria, of which 29.1% were falciparum malaria, 70.3% vivax malaria and only 0.6% mixed species (SIVEP-Malária, 2015).

Population and sampling

Participants were sourced from malaria cases diagnosed using passive case detection at health centers in Mâncio Lima in 2012 and 2013. Patients with malaria symptoms seeking diagnosis were first submitted to a thick blood smear by health agents. Patients who tested positive were asked to participate in the study,

and if they agreed to an interview and blood sampling were included in the study. For minors, both written parental/guardian consent and verbal patient's consent was obtained. Participants (or their parents/guardian) were interviewed using a structured questionnaire that included demographic variables (e.g. gender and age), duration of symptoms, and number of previous malaria episodes.

Thick blood smears were stained with Giemsa, and according to the Ministry of Health guidelines (Ministério da Saúde, 2005), at least 100 fields were examined under 700 times magnification for malaria parasites by two experienced local microscopists, using a semi-quantitative analysis of parasitemia (<200 parasites/ mm^3, 200-300 parasites/mm^3, 301-500 parasites/mm^3, 501-10,000 parasites/mm^3, and >10,000 parasites/mm^3).

Venous blood was collected in sterile vacuum tubes with EDTA. Samples were centrifuged, and blood was separated and stored in 20°C until tested. Blood samples were also examined using nested PCR-based amplification of a species-specific segment of the 18S rRNA gene of human malaria parasites, as described by Kimura et al. (1997) with modifications by Win et al. (2002). DNA templates for PCR amplification were isolated from 200 μL of whole blood (whenever available) using Macherey–Nagel genomic DNA extraction kit for tissue (NucleoSpin Tissue).

Anemia diagnosis: Blood samples were tested for quantification of hemoglobin using a portable hemoglobin Hemocue (Ängelhom, Sweden) immediately after blood collection. Anemia was defined as recommended by the World Health Organization (2008): children between 6-59 months old, Hb <11g/ dL; children between 5-11 years old, Hb<11.5g/dl; children between 12-14 years, Hb<12.0g/dL; women older than 15 years and not pregnant, Hb<12 g/dL over 15 years and pregnant women, Hb<11.0g/dL and men over 15 years, Hb<13g/dLl (WHO, 2008).

Treatment of malaria episodes

Patients were referred to treatment by authorized Ministry of Health workers, since drugs are only dispensed in the country by these officials. Patients with vivax malaria were treated with Chloroquine and Primaquine for 7 days and those with falciparum malaria were treated with a combined regimen of Mefloquine, Artesunate and Primaquine or of Artemeter and Lufemantrin (Coartem®), whenever possible, according to the current malaria therapy guidelines in Brazil (Ministério da Saúde, 2010).

Statistical Analysis

Data was entered using SPSS 19.0 software (SPSS Inc., Chicago, IL). Parasite loads were stratified into two levels: (a) <100 parasites in 100 fields (roughly corresponding to 1-300 parasites/ microliter of blood) and (b) >100 parasites in

100 fields (>300 parasites/ microliter of blood). Age was classified into less than 15 years and 15 years and above. The number of previous confirmed malaria episodes was divided per ≤8 and more than 8 episodes, corresponding to the median number or previous episodes of the subjects included in the study. The duration of symptoms in days was also divided per ≤4 and more than 4 days, corresponding to the median duration of days experiencing symptoms. The distribution of the independent variables was identified using Student's t-test to compare means and frequencies or proportions with α=0.05 critical level.

Ethical considerations

This study was approved by the Ethics Committee for Research on Human Beings at the Federal University of Acre (registration number 23107.016975/2011-28). Written informed consent was obtained from each participant or his/her parent prior to the study.

RESULTS

One hundred and twenty subjects with malaria were assessed, of which 58.3% male and 41.7% female. About 20.8% of the patients were younger than 15 years. The average age of the participants was 28.12 years (standard deviation of 15.80 years) and median 25 years. Approximately 97.5% of the participants resided in the municipality of Mâncio Lima, while 2.5% resided in neighboring municipalities (Rodrigues Alves and Cruzeiro do Sul).

Of the 120 cases, 81.7% were infected with *P. vivax*, 17.5% with *P. falciparum* and 0.8% with mixed infections (*P. vivax* and *P. falciparum*). About 60% of the patients had parasitemia equal to or less than 300 parasites/mm^3 (Table 1). The average number of malaria episodes in the previous year (2011) was 1.84 episodes (minimum was zero and maximum was 12 episodes in the year). The average number of episodes in lifetime was 11.39 (minimum of zero and maximum of 50); 43.5% of subjects had had more than 8 malaria episodes in their lives. Regarding symptoms, 61.2% had up to four days of symptoms and the others had experienced more than four days of symptom evolution.

There was a 25% prevalence of anemia in patients with malaria. An association was noted between anemia and the female gender (p=0.005, Fisher's exact test) and duration of symptoms longer than four days (p=0.025, Fisher's exact test). When the analysis was stratified by gender, it was observed that in males this association remained significant (p=0.041), but for females there was no longer an association between anemia and duration of symptoms (p = 0.310) (Table 2).

There was no association of anemia with type of parasitaemia infection, formation of gametocytes, age, number of malaria episodes throughout life or the previous year, or with the length of residence in the town.

Table 1. Characteristics of the study population

Variables (n = 120)	n	(%)
Gender		
Female	50	41.7
Male	70	58.3
Place of residence		
Mâncio Lima	117	97.5
Rodrigues Alves	1	0.8
Cruzeiro do Sul	2	1.7
Type of malaria		
P. vivax	98	81.7
P. falciparum	21	17.5
Mixed malaria	1	0.8
Parasitemia		
< 300 parasites/mm^3	48	40.0
>= 300 parasites/mm^3	72	60.0
Duration of symptoms*		
Up to 4 days	71	61.2
More than 4 days	45	38.8

*n= 116

Table 2. Factors associated with anemia

	Anemia			
Variables (n= 108)	n	Yes (%)	No (%)	p value
Gender				
Female	50	63.3	34.4	p= 0.005**
Male	70	36.7	65.6	
Duration of symptoms				
0 to 4 days	63	36	60.7	p= 0.025*
More than 4 days	51	64	39.3	
Duration of symptoms in males				
0 to 4 days	40	25	64.4	p= 0.041**
More than 4 days	27	75	35.6	

* Chi-square test, ** Fisher's Exact Test

DISCUSSION

The incidence of malaria in patients evaluated in Mâncio Lima is slightly lower than that found in some other studies conducted in the country. The prevalence of anemia in malaria in Brazilian patients varies between 36% and 82.6% in some studies. Fernandes et al (2008) reported 36% prevalence of anemia in the general population of patients with malaria. Cardoso et al (1992) reported a 44.4% prevalence, being higher in children and infants with malaria. Ventura et al (1999) found 82.6% of anemia in children and adolescents in the eastern Amazon, and anemia was associated with longer lapses between the onset of symptoms and diagnosis of the disease.

In this study, only 20.8% of patients were younger than 15 years and this may explain this difference in the prevalence of anemia. Another possible cause for the low prevalence of anemia in this study is the short duration of the disease (less than 4 days in 61.2% of patients) with early treatment of malaria, reducing complications (Ministério da Saúde, 2010), and the low incidence of malaria falciparum (17.5%), usually more often associated with anemia (Souza et al., 2012; Ronald et al., 2006).

Ronald et al. (2006) investigated the coexistence of malaria and anemia in 296 children from two Kumasi communities in Ghana. Anemia prevalence was 66.2% and 34.5% in Manhyia and Moshie Zongo, respectively. There was a significant association between anemia and falciparum malaria, communal residence (Moshie Zongo) and male gender. About 16.5% of the anemia in this population could be attributed to falciparum malaria.

Sousa-Figueiredo et al. (2012) conducted a cross-sectional study in northern Angola with 1,237 preschool children (0-5 years), 1,142 school-age children (6-15 years) and 960 women and found a 56.9% prevalence of anemia among children under five years of age and 44.5% and 44.3% among pregnant and non-pregnant women, respectively. The occurrence of anemia in children was strongly associated with infection by *Plasmodium* spp. and gender, with girls being less likely to be anemic than boys.

Despite the association of anemia to malaria in several cross-sectional studies, it is not possible to confirm whether the development of anemia is exclusively due to infection by *Plasmodium* or if there are overlapping factors such as nutritional deficiency (Cardoso et al., 1992; Katusuragawa et al., 2009; Noronha et al., 2000), intestinal parasites (Ventura et al., 1999; Cardoso et al., 1992) and precarious health conditions (Katusuragawa et al., 2009), since these factors are also present in malaria patients living in low socioeconomic conditions (da Silva-Nunes et al., 2007). Stoltzfus et al. (1997) verified that only 10% of the anemia in schoolchildren in two islands in Zanzibar off the coast of Tanzania could be attributed to malaria, and hookworm infection contributes to up to 25% of cases of anemia. Brooker et al. (1999) demonstrated similar contributions of malaria (18%) and hookworm infection (14%) in pre-school children with anemia in Kenya.

As for the relationship between gender and anemia, some studies show a higher prevalence of anemia in male children with malaria (Ronald et al., 2006) and in adult men (Souza-Figueiredo et al., 2012). Katsuragawa et al. (2009) identified higher frequency of malaria and anemia among men living in malaria endemic areas in the Brazilian Amazon since they are more exposed to malaria due to their work activities. On the other hand, the higher prevalence of anemia among women noted in Mâncio Lima has been reported by other studies in the Americas (Katsuragawa et al., 2009; Perez Mato et al., 1998). A possible explanation is that women are a risk group for anemia, regardless of malaria (Fabian et al., 2007; Lopes et al., 1999), and could therefore already have anemia or hemoglobin levels closer to normal limits before the Plasmodium infection took place, resulting in a higher incidence of anemia following rupture of the infected erythrocyte. Also, the fact that the duration of the symptoms had only been associated with anemia in males reinforces the hypothesis that in women anemia is a pre-existing condition.

Some studies show an association between anemia and parasitaemia in malaria (Noronha et al., 2000; Oliveira et al., 2004), while in other studies, this association was not confirmed (Fernandes et al., 2008). In the present study no association between parasitaemia and anemia was found.

As for the duration of malaria hemolytic anemia, Cardoso et al. (1992) reports a downward trend in the prevalence of anemia as time increases after the last malarial episode, especially after a period of three months, showing that anemia as a result of malaria tends to be an acute event, with fast recovery once malaria is treated.

Due to the seasonal nature of malaria, as well as genetic and socioeconomic variations in endemic populations, it is difficult to compare studies carried out in different regions of the world, limiting the comparison of such results. In addition, the cross-sectional nature of this study prevents verification of the causal relationship between malaria and anemia. Cohort studies in the Brazilian Amazon are needed for further clarification on the effect of malaria on hemoglobin levels and duration of these changes.

Malaria in Brazil is still a public health problem, especially in the Amazon. The prevalence of anemia and associated factors for patients diagnosed with malaria may be related not only to this infection, but also to the presence of previous or overlapping diseases. The diagnosis and early treatment of malaria should be instituted in order to reduce the incidence of infection, possible complications and severe disease. The prophylaxis and treatment of anemia in malaria endemic areas may also contribute to the reduction of severe cases of malaria and anemia, especially in children and pregnant women.

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