Occurrence of Intestinal Parasite-induced Anaemia and Its Association with Thrombocytosis: A Study from Riverside Communities in the State of Pará-Brazil

Fernanda Gomes de Souza¹, Thaís Luiza de Almeida Correa Lima¹, Érica dos Santos Sarges¹, Eduardo Dias de Almeida¹, Janaina Miranda Bezerra², Marly de Fátima Carvalho de Melo³ and Carolina Heitmann Mares Azevedo Ribeiro⁴*

¹Faculty of Pharmacy, Federal University of Pará, Belém, PA, Brazil.  
²Federal University of Maranhão, Imperatriz, MA, Brazil.  
³Program Luz na Amazônia, Federal University of Pará, Belém, PA, Brazil.  
⁴Laboratory of Hematology, Pharmaceutical Science Post-graduation Program Faculty of Pharmacy, Federal University of Pará, Belém, PA, Brazil.

Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

ABSTRACT

Aims: The objective of this study was to verify the occurrence of anaemia associated with intestinal parasites and their involvement in anemic patients’ platelet levels.

Study Design: Population-based descriptive cross-sectional study.

Place and Duration of the Study: School of Pharmacy, at Federal University of Para, and Program Lights in the Amazon, between May/2013 and June/2015.

*Corresponding author: E-mail: carolmheitmann@hotmail.com;
Methods: The participants were 114 individuals from riverside communities, which have been subject to blood count (automated methodology, using the hematology analyzer Sysmex XE2100) and parasitological examination (qualitative method of Lutz or Hoffman, Pons and Janer).

Results: The prevalence of anaemia in the individuals studied was 41.23% (47). Of these, 34.04% (16) had microcytosis and hypochromia. There was positive diagnosis of intestinal parasites in 84.21% (96) of the total population surveyed, with a prevalence of 95.74% (45) and 76.11% (67) for the anemic and non-anemic groups, respectively. Significant association was found between anaemia and parasitic diseases (p = 0.0102). The anemic group differed significantly from the non-anemic group in relation to platelet count (p = 0.0242). We also observed a higher incidence of thrombocytosis in anemic individuals, which corresponded to 14.89%, while thrombocytosis in the non-anemic group occurred in 4.26% of the individuals.

Conclusion: The prevalence of intestinal parasites and anaemia detected in this study confirms that these are important public health problems in the surveyed riverside communities. The occurrence of anaemia in individuals infected with parasites was significantly higher than in the non-infected group, which makes intestinal parasitosis a determinant and aggravating factor for anaemia. In addition, the higher incidence of thrombocytosis in anemic individuals was accompanied by a higher blood count.

Keywords: Anaemia; platelets; intestinal parasitosis; riverside communities.

1. INTRODUCTION

According to the World Health Organization (WHO) anaemia is defined as "a state in which blood concentration of hemoglobin is abnormally low in consequence to the lack of one or more essential nutrients in someone's diet, whatever is the origin of such lack" [1]. The United Nations Children's Fund (UNICEF) estimates that 90% of all types of anaemia in the world are related to iron deficiency [2]. In addition, they estimate that the lack of this element reaches four billion individuals and that iron deficiency anaemia affects more than two billion people worldwide, especially in developing countries and low-income populations [3].

In addition to the inadequate intake of nutrients, intestinal parasites have been considered important factors in the etiology of nutritional anaemia, representing a serious public health problem of global nature. In Brazil, these diseases occur in different regions of the country, whether in rural or urban areas and in different age groups. These conditions are correlated with lower socioeconomic status and poor sanitation conditions, representing a scourge, particularly for the poorest populations [4,5]. Medical literature reports that, in a state of anaemia due to iron deficiency, there is an increase in platelets. Research on platelet parameters in 86 women aged between 16 and 70 years with iron deficiency anaemia, thrombocytosis and thrombocytopenia were observed in 27.9% and 2.3%, respectively. The platelet count was increased when the serum iron saturation, ferritin and MPV were decreased. Platelet counts and iron saturation index were the most affected factors, also suggesting a relationship between decreased levels of iron in the tissues and the behavior of platelets in addition to the stimulus of low iron saturation in megakaryocytogenesis. Another factor to be considered is the possibility of iron having an inhibitory effect on platelet count [6].

According to some studies, compatible explanation for the increase in platelet levels for iron deficiency anaemia would be the rapid maturation time and / or increase of polyploid megakaryocytes resulting in increased production of platelets. This suggests that thrombocytosis deficiency anaemia can result due to changes in the level of pluripotent "stem cells", wherein the stimulation of erythropoietin maturation could result in a shunt in the "stem cells" in parallel with the cell line [7]. Other studies documented in the literature propose different mechanisms to justify the platelet levels increase in iron deficiency anaemia.

Platelets are cytoplasmic coreless fragments present in blood and produced from megakaryocytes in the bone marrow. From the total platelets present in the human body, 70% are present in the circulation and 30% in the spleen, remaining in circulation for an average of ten days, when they are withdrawn by the reticuloendothelial cells of the spleen and liver [8-10].
The reference value for platelets under normal conditions exhibit a great variation in the literature. Considered to be a value around 150,000 to 450,000 platelets per microliter of blood. The reference values for platelet counts are similar in both sexes, and independent of age [11-13]. They play important roles in primary hemostasis (adhesion and aggregation function), and secondary hemostasis (correlate with coagulation factors), are involved in thrombosis mechanisms and are causes of diseases, both by decreasing their number (thrombocytopenia), and by its increase (thrombocytosis) and change of function [11].

Therefore, the present study aimed to verify the prevalence of intestinal parasites-induced anaemia and its association with thrombocytosis in residents from two riverside communities in the state of Pará-Brazil.

2. MATERIALS AND METHODS

2.1 Design, Study Population and Data Collection

This study has a transversal character. The epidemiological survey was conducted on the prevalence of anaemia and parasites and its interference in platelet parameters in subjects of both sexes aged 1-76 years-old, living in riverside communities located in the northeast of the State of Para: Genipaúba and Furo of Aurá. This study was carried out as a part of the Program Light in the Amazon, which is a partnership between the Brazilian Biblical Society (SBB) and the Federal University of Para (UFPA). This program has the support of volunteers, and develops actions for the promotion of public health in riverside communities.

The study included 114 individuals. The period of data collection was conducted from April 8 to October 7, 2014. Socioeconomic conditions were not evaluated.

2.2 Inclusion and Exclusion Criteria

The study included all subjects coming from the two communities studied who have undergone complete blood count (CBC) test and parasitological examination. The study excluded individuals who only did the blood test or stool examination. Compromised samples for analysis result from traumatic collection were excluded.

2.3 Ethical Aspects

The research protocol was reviewed and approved by the Ethics Committee of Research in Humans (CEPH) from the Health Sciences Institute (ICS) of the Federal University of Para on November 25, 2015, with the registration number 1337273.

2.4 Laboratory Analysis

The biological material (blood) to perform the blood count was collected by a qualified clinical analysis technician and trained undergraduate students. This collection was made by venipuncture in tubes containing EDTA (ethylenediaminetetraacetic acid) as an anticoagulant.

Subsequently, the samples were placed in Styrofoam in proper temperature and sent to the hematology laboratory at the School of Pharmacy-UFPA and the Evandro Chagas Institute for the proposed analysis. The samples were analyzed by automated methodology, using the hematology analyzer Sysmex XE2100 (Sysmex, Kobe, Japan), in which we evaluated the following parameters: red blood cells, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), red cell volume distribution width (RDW) and total leukocytes. In view of possible cellular abnormalities, such as poikilocytosis, blood film was performed on all samples using the panoptic staining which was subsequently submitted to optical microscopy analysis for the visualization of cellular elements, in order to characterize anaemia.

Platelet levels were also determined in the hematology analyzer. Platelet counts (PLT) were obtained by a technique called direct current (DC), where the cells are sorted by volume. The remaining platelet parameters such as mean platelet volume (MPV), platelet distribution of the population (PDW) and plachetocrit (PCT) were analyzed by flow cytometry technique using monoclonal antibodies and thiazole orange (TO).

To verify the presence of intestinal parasites, possible determinants of anaemia, stool samples were collected and subjected to the qualitative method of Lutz or Hoffman, Pons and Janer (spontaneous sedimentation), enabling the verification of eggs and larvae of helminths and
protozoan cysts in the analyzed feces. Approximately 2 g of feces in a barrel bottle or plastic disposable beaker were dissolved in 5 ml of water with the aid of a disposable popsicle stick. Another 20 ml of water was added and the suspension was strained (to do this, we used surgical moistened gauze, folded into four, and placed in a small plastic strainer) in a tapered cup of 200 ml capacity. Debris retained on the gauze were washed with an additional 20 mL of water. After 24 hours, the supernatant was discarded carefully, and a portion of homogenized slurry was collected, stained with Lugol and covered with a coverslip for visualization of the parasite, using small increase microscopic lens (10x) and reduced light intensity. Confirmation of the parasites was made with the use of high-power objective lens (40x).

2.5 Anaemia Diagnosis

Individuals were considered anemic when they had hemoglobin levels below the recommended by WHO. According to WHO (2001) hemoglobin values for 6 months to 4 years-old children is 11.0 g/dL, 11.5 g/dL for children 5 to 11 years-old, for children 12 to 14 years-old and for women over 15 years-old the ideal amount of hemoglobin is 12.0 g/dL, and for men over 15 years-old the figure is 13.0 g/dL [14].

2.6 Statistical Analysis

Study’s participants were divided into two groups: anemic and non-anemic. Data from all participants were used in order to verify the association between anaemia and parasitosis. Only data from infected individuals, regardless of the presence of anaemia, were used to assess the correlation between groups in regards to hematological parameters and platelet levels. The results of descriptive analysis of all parameters were expressed as a ratio. The results were dichotomized or expressed as mean ± standard deviation for groups comparison. For parametric variables we used the Student t test and for non-parametric the Odds Ratio tests. In all analyzes performed, analyzed parameters were considered statistically significant when the P value was <0.05. Data were tabulated using Microsoft Excel® 2013 and the statistical analysis was carried out with the GraphPad PRISM® 6.0 software and is presented in tables and/or graphics.

3. RESULTS AND DISCUSSION

Of the 114 individuals analyzed, most were women, corresponding to 58.77%. The prevalence of anaemia in individuals studied was 41.23%, being 46.81% males and 53.19% females.

As for the evaluated hematological parameters, it can be seen in Table 1 that the anemic infected group differed significantly from the infected non-anemic group on the following parameters: Hb, HCT, MCV, MCH, MCHC and RDW.

Table 1. Average and standard deviation of hematological indexes of the groups evaluated

|                        | Infected anemic (n = 45) | Infected non-anemic (n = 51) | p value |
|------------------------|--------------------------|-----------------------------|---------|
| Hb (g/dl)              | 11.15±1.09               | 13.01±0.97                  | .0001   |
| HCT (%)                | 34.57±2.94               | 39.44±2.75                  | .0001   |
| MCV (fl)               | 76.99±10.19              | 80.92±3.96                  | .0126   |
| MCH (pg)               | 24.81±3.50               | 26.69±1.39                  | .0006   |
| MCHC (g/dl)            | 32.18±1.14               | 33.00±0.72                  | .0001   |
| RDW (%)                | 15.20±1.99               | 13.75±0.79                  | .0001   |

Significance level: p < 0.05. Student t test

Nutritional anaemias are the most prevalent in developing countries, being iron deficiency anaemia the most common, often appearing with microcytosis and hypochromia. Data from the literature were considered to determine the presence of microcytosis and hypochromia. A sample would be considered microcytic with a MCV below 80 fl, and hypochromic with MCH below 24 pg. Of all the individuals who had anaemia, 59.58% had microcytosis, 34.04% had hypochromia and 34.04% had both microcytosis and hypochromia.

In this study, we observed the positive diagnosis of intestinal parasites in 84.21% of the total population surveyed, with a prevalence of 95.74% for the anemic group and 76.12% for the non-anemic group, as shown in Fig. 1.

Statistical analysis revealed significant correlation between anaemia and intestinal parasitosis (p = 0.0102; OR = 7.059). Therefore, anaemia occurrence probability in parasite-infected individuals was approximately 7 fold higher than in the non-parasite-infected ones.
The Table 2 shows that polyparasitism 46.49% have higher expression than duo parasitism 26.32% and single parasitism 11.40% together.

**Table 2. Prevalence of single parasitism, duo parasitism and polyparasitism in the riverside population studied**

| Parasite infection   | N   | %    |
|----------------------|-----|------|
| Single parasitism    | 13  | 11.40|
| Duo parasitism       | 30  | 26.32|
| Polyparasitism       | 53  | 46.49|
| Negative             | 18  | 15.79|
| Total                | 114 | 100  |

The main parasite species found in this study are shown in Table 3. There was a higher frequency of parasitism caused by protozoa compared to those caused by helminths.

**Table 3. Intestinal parasite species distribution obtained in the parasitological analysis of the riverside communities studied**

| Parasite species         | N   | %    |
|--------------------------|-----|------|
| Helminths                |     |      |
| Trichuris trichiura      | 77  | 29.82|
| Ascaris lumbricoides     | 25  | 10.09|
| Hookworm                 | 18  | 7.34 |
| Protozoan                |     |      |
| Endolimax nana           | 48  | 18.81|
| Entamoeba coli           | 44  | 17.43|
| Iodameba butschlii       | 17  | 7.80 |
| Giardia lambia           | 13  | 5.96 |
| Entamoeba histolytica    | 6   | 2.75 |

Regarding individual parasite frequency, *Trichuris trichiura* (29.82%) was the most frequent in parasitological examinations, followed by *Endolimax nana* (18.81%), *Entamoeba coli* (17.43%), *Ascaris lumbricoides* (10.09%), *Iodameba butschlii* (7.80%), Hookworm (7.34%), *Giardia lambia* (5.96%) and *Entamoeba histolytica* (2.75%).

As for the evaluated platelet parameters, the anemic group differed significantly from the non-anemic group in relation to platelet count, but there was no significant difference between the two groups in terms of MPV, PDW and PCT as shown in Table 4.

**Table 4. Platelet indexes of the groups evaluated**

|                  | Infected anemics (n = 45) | Infected non-anemic (n = 51) | p value |
|------------------|---------------------------|------------------------------|---------|
| PLT (10⁹/L)      | 323.47±99.87              | 284.43±65.31                 | .0242   |
| PDW (fL)         | 14.31±2.26                | 14.65±1.89                   | .4304   |
| MPV (fL)         | 11.20±0.99                | 11.36±0.82                   | .3946   |
| PCT (%)          | 0.34±0.11                 | 0.3243±0.07                  | .4255   |

Cases of thrombocytopenia were not reported within the groups studied. A higher incidence of thrombocytosis was verified in 14.89% of the anemic individuals, while only 4.26% of the non-anemic individuals were diagnosed with thrombocytosis.

### 3.1 Discussion

Anaemia remains the most prevalent nutritional deficiency in the world, affecting 1.62 billion people or 24.8% of the population. According to the World Health Organization (WHO), Brazil is one among the Latin American countries where the prevalence of anaemia is considered a serious public health problem [15].

The present study found a significant prevalence of anaemia in the surveyed population, where more than 40% were anemic. These results are
similar to the ones found in another study of two Amazonian riverside communities, “São Luiz do Tapajós” (Tapajós River) and “Igarapé-Mirim” (Tocantins River), where hematometric indexes pointed out 40% and 25% of anaemia incidence in São Luiz do Tapajós and Igarapé-Mirim, respectively [16].

Group comparison of the erythrocyte parameters revealed a statistically significant difference for the values Hb, HCT, MCV, HCM, MCHC and RDW. According with the evaluation of these parameters, specifically Hb levels and MCV and HCM values, 34.04% of the surveyed were diagnosed with hypochromia and microcytic anaemia, revealing a significant incidence of this type of anaemia in our study. Also, the degree of anisocytosis was higher in the anemic group.

Microcytic and hypochromic anaemias are the most prevalent in the world [17]. Several causes can lead to this situation, such as iron deficiency for hemoglobin synthesis, which is caused both by the absorptive or nutritional deficiency, as well as chronic blood loss. In addition, microcytic and hypochromic anaemias can also be caused by loss of iron supply for erythropoiesis, as occurs chronic diseases-related anaemia; quantitative genetic defects in the synthesis of globin chains, as in thalassemia; and defects in heme synthesis, as is the case for congenital sideroblastic anaemia [18]. Anisocytosis index is a distinguishing feature of microcytic and hypochromic anaemias. Thalassemia minor and chronic disease anaemia present homogeneous populations of red blood cells, while iron deficiency anaemia, presents heterogeneous populations [19].

Given the above outlined results and factors, it is suggested that anaemia in individuals surveyed is due to iron deficiency.

This work demonstrated a significant association between anaemia and intestinal parasitosis, as observed in other studies in the scientific literature. In a survey of 545 medical records from a laboratory in the central region of Florianópolis, between January 2000 and August 2001, 60 subjects were diagnosed with parasitosis-associated anaemia [20]. Another study, in Igarapé Miri (Pará State), 91 samples from five communities (Alto Anapu, Baixo Anapu, Cruzeiro, Jarimbu, Menino Deus) underwent parasitological analysis, which revealed a 94.5% incidence of parasitosis positive diagnosis. Association with anaemia was reported for 90.32% of the samples [21].

The most common parasites in the analyzed individuals were Trichuris trichiura, Entamoeba coli, Ascaris lumbricoïdes and Endolimax nana. Studies with the riverine population from Nova Olinda do Norte, Amazonas, Ascaris lumbricoïdes and Trichuris trichiura were the most common parasites among the elderly, with 35.2% and 15.9% prevalence, respectively [22].

The parasites Trichuris trichiura, Entamoeba coli and Ascaris lumbricoïdes are considered endemic in Brazil since they have wide geographic distribution and high prevalence. Parasites as Endolimax nana and Entamoeba coli, although not considered pathogenic, serve as parameters to measure the degree of fecal contamination that individuals are exposed to as they are commensal parasites in the human gut [23].

It’s important to highlight the 7.34% occurrence of hookworm in the studied population, as it is known that in cases of ancylostomosis anaemia is due to the hematophagism practiced by the adult forms of Ancylostomatidae worms [24]. These data corroborate the findings of Silva et al. [20], who reported the presence of Ancylostomatidae in 13.09% of their analyzed samples, which could have been higher have they used a more appropriate and specific method for the diagnosis of Ancylostomatidae worms.

Regarding platelet parameters, the present study did not find statistically significant differences between the groups for the following platelet parameters: PDW, MPV and PCT. However, a statistically significant difference in platelet count was observed. There is wide variation in the literature regarding the reference value for platelet count under normal conditions. It is estimated an approximate number of 150,000 to 450,000 platelets per mm³, which remains constant for each individual in physiological situations [25-27].

The increase in platelet count occurred with a higher incidence in the anemic group. In the anemic group 14.89% of the individuals showed thrombocytosis, whereas in the non-anemic group only 4.26% presented increased platelet count. These results corroborate the findings of Perdigão (2003), which in their platelet studies with 55 iron-deficient patients, found that 35% of them had a platelet count higher than 450,000 / mm³ when compared to the control group (277,000 / mm³ ±9.8), therefore, supporting the
correlation between iron-deficiency anaemia and increased platelet count [28].

Other studies documented in the literature also checked for the occurrence of thrombocytosis associated with iron-deficiency anaemia. Kadikoylu et al. [29] evaluated platelet parameters in 86 women with 16 to 70-years-old and diagnosed with iron-deficiency anaemia, and found a 27.9% and 2.3% incidence of thrombocytosis and thrombocytopenia, respectively. They also noted that platelet count was increased when serum iron saturation, ferritin and MPV were decreased and that platelet and iron saturation indexes were the most affected factors, which also suggests a relationship between decreased tissue iron levels and the behavior of platelets, in addition to the iron low saturation stimulus in megakaryocytopenosis [29].

Choi and Simone [30] induced iron-deficiency anaemia in rats and observed that both the production levels and count of platelets increased as the iron-deficiency anaemia progressed. Also, when they injected rats with iron, these parameters decreased within the next 72 hours, and an inverse relation between hematocrit and platelet count, which suggests that platelet homeostasis is highly influenced by iron-deficiency severity.

The occurrence of higher than normal blood count is a very common event that can be due to both physiological and pathological reasons. However, it's important to highlight that besides the increased thrombotic risk associated with high platelet count, decrease in antioxidant defenses during iron-deficiency anaemia can lead to an enhanced oxidative stress status, which in turn facilitates platelet aggregation [31]. Thus, the observed increase in platelet count during iron-deficiency anaemia can synergistically act to promote the formation of thrombi, particularly to the configuration of a subjacent atherosclerotic disease [32]. Therefore, thrombocytosis is relevant factor to take into account in cases of iron-deficiency anaemia.

Due to resource limitations, serum iron determination tests were not carried out in the present study as a way to provide a differential diagnosis of both microcytic and hypochromic anaemia, which can be caused by either iron deficiency or thalassemia. Nonetheless, erythrocyte heterogeneity in the microcytic and hypochromic anaemia group collaborates to the differentiation between iron deficiency and thalassemia, in which there is microcytosis with homogeneously small erythrocytes [33].

4. CONCLUSION

The results found in the present study allow for the conclusion that there is a significant prevalence of anaemia among the surveyed population. Also, intestinal parasitosis seem to be an important determinant or aggravating factor in cases of anaemia. Furthermore, we also found a higher incidence of thrombocytosis in the anemic individuals. Nonetheless, more studies to further investigate such association are needed.

CONSENT

All authors declare that a written informed consent was obtained from the patients (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee. The research protocol was analyzed and approved by the Ethics Committee for Human Research of the Institute of Health Sciences of Federal University of Pará, in November 09th, 2014, according to the number of protocol 156/08 CEP-ICS/UFPA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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