Research Article

Study of prevalence of different species of malarial parasites and comparison of hematological parameters in different malarial parasite species

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Received May 16, 2015. Accepted June 1, 2015

Abstract

Background: Malaria is one of the major public health problems. Currently, almost 100 countries or territories in the world are considered to be affected by malaria, with Africa and South of Sahara accounting for half of them. The estimation of WHO is that 1.7 to 2.5 million deaths and 300 to 500 million cases of malaria occur each year globally. The disease is distributed in all parts of India.

Objective: To find out the prevalence of different species of malaria in Surendranagar district (Gujarat, India) and comparison of different hematological parameters between the species. Basic procedure includes all patients revealing malaria infection by any species.

Material and Methods: Hematological parameters such as hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), total leukocyte count (TLC), platelet count, and red cell distribution width (RDW) were determined by using automated cell counter, and peripheral smear examination for malarial parasite was taken as gold standard for diagnosis of malaria.

Result: Of the 129 patients, 60 patients revealed *Plasmodium falciparum* malaria and 69 patients revealed *Plasmodium vivax* malaria; other species has not been identified in this region. Anemia (Hb < 10 g/dL, $p = 0.00$), platelet count (<50,000 cells/mm^3, $p = 0.01$), and PCV(<35%, $p = 0.00$) were the best favoring parameters of *P. falciparum* when compared with *P. vivax*.

Conclusion: We concluded that prevalence of *P. vivax* was higher than *P. falciparum* in southeast region of Saurashtra and that *P. falciparum* compared with *P. vivax* can cause significant hematologic changes with high frequency of thrombocytopenia, anemia, and decreased PCV. The blood changes are so characteristic that the diagnosis of malaria should always be considered in the presence of aforementioned findings.

KEY WORDS: Malaria, prevalence, hematological parameters

Introduction

Malaria is a protozoan disease transmitted by the bite of infected female Anopheles mosquito. Since, many centuries, malaria is known to human beings, particularly Africa and Asia. Although there is an increase in knowledge, malaria remains to be a major reason of noteworthy morbidity and mortality worldwide.[1] Malaria is one among the most widespread human infections in the world. More than 40% population of the world live in malaria endemic area, and it is estimated by World Health Organization (WHO) that 1.7 to 2.5 million deaths and 300 to 500 million cases of malaria occur each year globally.[2] Of about 1.4 billion people residing in 11 countries (land area, 8,466,600 km^2; i.e., 6% of global area) of the southeastern Asian region of WHO, almost 1.2 billion are prone to the infection of malaria, of whom most belong to India.[3] Nonetheless, the contribution of the southeast Asia

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2015.16052015350	

region was only 2.5 million cases to the worldwide burden of malaria. Of this, 76% of the total cases occur in India.[4]

Distribution of malaria is different in different states of India. The malariometric index used to represent malaria cases per thousand people is known as the annual parasite incidence (API). As per the National Vector Borne Disease Control Program (NVBDCP), India, 2004, in most of India, the API was <2; the regions with >5 API were in the states of Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chattisgarh, Jharkhand, Orissa, and Northeastern states (data source: NVBDCP, India, 2004).

In severe malaria (parasitemia > 5%), the mortality is usually high (20%). The reported hematological aberrations that habitually occur with malaria include anemia, thrombocytopenia, lymphocytosis, and rarely disseminated intravascular coagulation.[5] The aim of this study was to know the prevalence of different species of malaria and to evaluate whether any difference in hematological parameters in different species of malaria.

Materials and Methods

The study was conducted from January 2009 to June 2010 in Hematology Laboratory, CU Shah Medical College and Hospital, Surendranagar, Gujarat, India. A total of 129 consecutive patients of all ages and sex experiencing malaria were included in this study. They presented to CU Shah Medical College and Hospital, Surendranagar, with fever. The diagnosis of malaria was confirmed by thin and thick blood films stained with Leishman's stain for malaria parasite. The species were identified and recorded. The study included clinical history and hematological changes in relation to types of malaria. Patients showing clinical history and/or findings of chronic liver disease, drug intake, any bleeding disorder, thrombocytopenia, or conditions that might change hematological parameters were excluded from this study.

Complete blood counts were performed using an automated CELL DYN–1700 machine. All malaria-positive smears were reviewed for confirmation, identification of species, and review of smear for platelet count and other hematological changes. Null hypothesis for this study is that hematological parameters between different species of malaria are same. Reference ranges for thrombocytopenia mild (<1,50,000–50,000 cells/mm^3) and moderate to severe (<50,000 cells/mm^3), criteria for hemoglobin (<10 g/dL), packed cell volume (PCV) (<35%), mean corpuscular volume (MCV) (<80 fL), RDW (>15%), and total leucocyte count (TLC) (>10,000 cells/mm^3) were studied. Statistical analysis included *t* test and χ^2 test. A *p* value of <0.05 was taken as significant for all statistical analysis.

Results

A total of 129 patients fulfilled the inclusion criteria. *Plasmodium vivax* malaria was more common than *Plasmodium falciparum*: 69 (53.48%) and 60 (46.52%), respectively, while other malaria species have not been identified in this region. The most common presenting symptoms of patients were high grade fever, high grade fever with chills or rigors, headache, vomiting, and loss of appetite. Majority of patients were working as laborer or coming from low socioeconomic status.

As shown in Table 1, of the 60 patients infected by *P. falciparum*, 36 patients revealed Hb < 10 g/dL, and of the 69 patients infected by *P. vivax*, 16 patients showed Hb < 10 g/dL (p = 0.000021, which was <0.05), which was statistically significant. It proves our hypothesis that hematological parameter Hb < 10g/dL is not similar in both species: when Hb < 10 g/dL in the patients with clinical symptoms, it is more in favor of diagnosis of *P. falciparum*.

As shown in Table 1, of the 60 patients infected by *P. falciparum*, 26 patients showed platelet count < 50,000 cells/mm^3, and of the 69 patients infected by *P. vivax*, 16 patients showed platelet count < 50,000 cells/mm^3 (p = 0.01, which was <0.05), which is statistically significant. It proves our hypothesis that hematological parameter platelet count <50,000 cells/mm^3 is not similar in both the species: when the platelet count is <50,000 cells/mm^3 in the patients with clinical symptoms, it is more in favor of diagnosis of *P. falciparum*.

As shown in Table 2, of the 60 patients with *P. falciparum* infection, 54 patients showed PCV < 35%, and of the 69 patients with *P. vivax* infection, 50 patients showed PCV < 35%, (p = 0.00, which is <0.05), which is statistically significant. It proves our hypothesis that hematological parameter PCV < 35% is not similar in both the species: when PCV is <35% in the patients with clinical symptoms, it is more in favor of diagnosis of *P. falciparum*.

Discussion

In India, about 70% of the infections are reported to be caused by *P. vivax*, 25%–30% by *P. falciparum*, 4%–8% by mixed infection, and 1% by *P. malariae*.[6] In our study, *P. vivax* malaria was more common than *P. falciparum* infection: 69 (53.48%) and 60 (46.52%) respectively, while other malaria species have not been identified in this region. The reported hematological aberrations that habitually occur with malaria include anemia, thrombocytopenia, lymphocytosis, and rarely disseminated intravascular coagulation.[4] But, specific changes may vary with level of malaria, background hemoglobinopathy, nutritional status, demographic factors, and malaria immunity.[7]

In this study, we observed several significant changes involving hemoglobin, platelet count, and PCV. Anemia was present in 52%; of the 60 patients infected by *P. falciparum*, 36 and, of the 69 patients infected by *P. vivax*, 16 showed Hb < 10 g/dL, with *p* value of 0.000021, which is <0.05. Of them, majority of the patients showed normocytic normochromic type, a finding that is concordant with the reports of Facer and Beale et al.[4,8] Traditionally, *P. falciparum* infection has been considered to result in anemia more frequently and

Table 1: Hematological changes in *P. falciparum* and *P. vivax* malaria

Hematological parameters	*P. falciparum*, *n* (%)	*P. vivax*, *n* (%)
Hemoglobin (g/dL)		
<10	36 (24.18)	16 (27.81)
>10	24 (35.81)	53 (41.18)
Platelet count (cells/mm^3)		
<1,50,000	58 (57.67)	66 (66.32)
>1,50,000	2 (2.33)	3 (2.67)
<50,000	26 (19.53)	16 (22.46)
>50,000 cells	34 (40.46)	53 (46.53)
Packed cell volume (PCV) (%)		
<35	54 (52.55)	59 (60.44)
>35	6 (7.44)	10 (8.55)
Mean corpuscular volume (MCV) (fL)		
<80	29 (26.51)	28 (30.48)
>80	31 (33.48)	41 (38.51)
Red cell distribution width (RDW)		
>15% CV	43 (43.25)	50 (49.74)
<15% CV	17 (16.74)	19 (19.25)
Total leukocyte count (cells/mm^3)		
>10,000	14 (10.98)	9 (12.01)
4,000–10,000	39 (42.01)	49 (45.98)
<4,000	7 (8.37)	11 (9.62)
>4,000	53 (51.62)	58 (59.37)

Table 2: Different hematological parameters with *p* value

Hematological parameters	*P. falciparum* (*n* = 60)	*P. vivax* (*n* = 69)	*p*
Hemoglobin < 10 g/dL	36	16	0.00
Platelet count < 50,000 cells/mm^3	26	16	0.01
Platelet count < 1,50,000 cells/mm^3	58	66	0.75
PCV < 35%	54	59	0.00 (*t* test)
MCV < 80 fL	29	28	0.37
RDW > 15% CV	43	50	0.92
Total leukocyte count > 10,000 cells/mm^3	14	09	0.15
Total leukocyte count < 4,000 cells/mm^3	7	11	0.49

with more severe degree than infections caused by *P. vivax*.[9] The pathogenesis of anemia in malaria is extremely complex, multifactorial, and not completely understood. It is thought to result from a combination of hemolysis of parasitized red blood cells, accelerated removal of both parasitized and innocently unparasitized red blood cells, depressed and ineffective erythropoiesis with dyserythropoietic changes, and anemia of chronic disease.[10,11]

Other factors contributing to anemia in malaria comprise reduced red blood cell deformability, splenic phagocytosis, and/or pooling; hence, they have an elevated rate of clearance from the circulation.[12] Tumor necrosis factor-alpha has also been implicated and may cause ineffective erythropoiesis.[13]

Different types of malaria show variable degree of reduction in circulating platelet count consistently,[14] but severe thrombocytopenia is quite rare in *P. vivax* malaria.[13] In our study, 96% of the patients with malaria developed thrombocytopenia, a percentage higher than reported by other investigators such as Robinson et al. (71%),[15] Rodriguez-Morales et al. (58.97%),[16] and Bashwari et al. (53%).[17] There was no significant difference in the incidence of thrombocytopenia between *P. falciparum* (96%) and *P. vivax* (95.6%), but severe thrombocytopenia was more common in *P. falciparum* (43.3%) than *P. vivax* (23.2%), and this percentage is higher than that reported by other investigators.[18] Patient who developed thrombocytopenia

because of malaria rarely bleed what ever the degree of reduction in platelets count.[19]

Although no particular mechanism of thrombocytopenia in malaria has been proposed, researchers have reported reduced thrombopoiesis could be a causative factor for thrombocytopenia in the infections of *P. falciparum* and *P. vivax*. However, bone marrow examination usually shows normal or increased megakaryocytes.[4] Peripheral destruction caused by *P. falciparum* involves production of immune complexes by malarial antigens that result in the sequestration of the damaged platelets by macrophages in the spleen; however, this mechanism is yet to be appropriately assessed in *P. vivax* malaria.[8] Some workers have suggested Disseminated intravascular coagulation (DIC) has been proposed as a main mechanism by some researchers, but not many have found any indication or have rarely observed DIC in any of their patients, even in those with severe thrombocytopenia.[20] The spleen has been implicated as a site of excess sequestration. Nonetheless, only splenomegaly alone cannot be the mechanism because many patients reveal thrombocytopenia at the early course of the infection itself even before the development of splenic enlargement.

Hypersensitive platelets are seen in acute malaria infection, and many platelet-specific proteins such as beta-thromboglobulin and platelet factor 4 are elevated in their concentrations. Production of thromboxane A2 and prostacyclin also increased. In addition, it has been proposed that these hypersensitive (hyperactive) platelets will increase the responses of hemostatic which can be the reason for rare occurrences of why bleeding episodes in acute malarial infections, in spite of the noteworthy thrombocytopenia.[17]

The observed significantly lower PCV and higher potassium (K+) in the *P. Falciparum*-infected subjects than the normal subjects can be credited to enormous destruction of the infected erythrocytes by the organisms. This will in effect lower the PCV and increase the level of K+ in plasma.[21–25]

On the contrary to some studies that revealed leucopenia to be a common outcome in both nonimmune and semi-immune patients.[4] We observed normal leukocyte count in 68% of the patients. No significant changes in other hematological parameters have been noted.

Conclusion

We concluded that prevalence of *P. vivax* is higher than *P. falciparum* in southeast region of Saurashtra and that *P. falciparum* compared with *P. vivax* can cause significant hematologic changes with high frequency of thrombocytopenia, anemia, and decreased PCV. The blood changes are so characteristic that the diagnosis of malaria should always be considered in the presence of aforementioned findings.

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How to cite this article: Gondaliya S, Makwana H, Lakum N, Agnihotri A. Study of prevalence of different species of malarial parasites and comparison of hematological parameters in different malarial parasite species. Int J Med Sci Public Health 2015;4:1697-1701

Source of Support: Nil, **Conflict of Interest:** None declared.