Brief Original Article

Hide and seek: hematological aspects of malaria – a developing country perspective

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Abstract

Introduction: Malaria, a major problem in tropical climates, presents with various hematological aberrations. We aimed to assess whether complete blood indices would increase the probability of malaria in patients with acute febrile illness.

Methodology: Between February 2009 and December 2010, we conducted a study involving 824 consenting consecutive patients older than 12 years with a confirmed diagnosis of malaria based on thick and thin blood films. A detailed history and physical examination were completed on all patients before inclusion. Complete blood counts and detection of *Plasmodium* species were also performed as well as liver function tests, prothrombin time, reticulocyte counts, and parasite load. All data was analysed using SPSS 16.0 and percentages were calculated.

Results: Out of 824 patients, 616 (75%) were male and 208 (25%) were female with an age range of 18 to 55 years (33.2 ± 8.3). Out of 87% thrombocytopenic patients, 66% were affected with *Plasmodium falciparum* and 21% with *Plasmodium vivax*. In patients with *P. falciparum*, thrombocytopenia was mild (16%), moderate (43%) and severe (7%), while in *P. vivax* patients thrombocytopenia was mild (10%) and moderate (9%). Thrombocytopenia was moderate in the mixed cases (2%). Anemia was seen in 71% and normal leucocyte counts were observed in 79% of the cases. Normal differential leucocytes counts were seen as follows: eosinophils in 80%, neutrophils in 93%, lymphocytes in 85%, monocytes in 97%, and basophils in 100%.

Conclusion: Blood indices should be included in patient evaluations as various hematological aberrances can lead to the diagnosis of malaria.

Key words: malaria; Pakistan; blood indices

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Introduction

Global efforts to control malaria in recent decades have made little progress, and it remains the most widespread arthropod-borne disease [1,2]. As its clinical features overlap with many other diseases it is often difficult to diagnose quickly. Malaria claims 1.1 to 2.7 million lives annually and over 40% of the world's population is at risk of contracting malaria, since they live in malaria endemic areas [3,4,5]. Pakistan has an incidence of one case per thousand population [6].

Resource-poor countries still rely on clinical diagnosis and presumptive antimalarial treatment has resulted in the emergence of chloroquine resistant species [7,8]. Although microscopy is the single most important diagnostic test, it requires trained individuals with technical expertise to perform and analyse the tests, and diagnostic aberrancies may result in tests performed by different individuals [8].Blood, the most easily accessible tissue of the body, can be used for rapid diagnosis of hematological changes which have been reported in settings where malaria is found [9].

The hematological changes previously reported in the literature include anemia, thrombocytopenia, atypical lymphocytosis, leucopenia, leukocytosis, neutropenia, neutrophilia, eosinophilia, monocytosis and rarely disseminated intravascular coagulation (DIC) [10]. We aimed to assess whether complete blood indices would increase the probability of malaria in patients with acute febrile illness. This approach can heighten the suspicion of malaria in tropical fevers, instigating a more meticulous quest for malarial parasites, thus saving time and money for both the physician and the patient.

Methodology

This observational study was conducted between February 2007 and February 2011 in the Department of Internal Medicine, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan. A total of 824 consenting consecutive patients were included in the study, who were either males or females older than 12 years of age with a confirmed diagnosis of malaria based on thick and thin blood films. According to our teaching hospital's policies, 12 years is the cutoff age for paediatrics patients. Patients with a concomitant proven hematological disorder due to secondary causes, such as chronic liver disease, drug use, or associated dengue fever, were excluded from the study as were patients already on antimalarial therapy, those with primary hematological disorders, and pregnant females with malaria. A detailed history and physical examination were performed for each patient. Fever patterns, presence of jaundice, hepatosplenomegaly, lymphadenopathy, skin petechial hemorrhages, chills and rigors, and urine color were specifically noted. Complete blood counts and detection of *Plasmodium* species were completedbefore patients were included in the study and liver function test, prothrombin time, reticulocyte count, and parasite load were completed for every included patient. Detection of fibrin degradation products (FDP) and D-dimer were also performed in some cases. We used only selected clinical and laboratory parameters while doing analysis. All data was analyzed using Statistical Package for Social Sciences version 16.0 (SPSS, IBM, Chicago, USA). Baseline hematological parameters such as hemoglobin, platelets, leukocyte counts, and their differential counts were classified into normal and pathological counterparts. Frequencies and percentages were calculated for the patients falling into each of these categories. The percentages of patients who presented with the clinical features were calculated and stratified according to the *Plasmodium* species. P-values were calculated by chi-square method. The most important hematological aberrations such as anemia and thrombocytopenia were compared among the three categories of malaria (*falciparum*, *vivax*, and mixed) and chi-square was used to calculate the p-values. The sensitivity, specificity, and predictive values were also calculated for anemia and thrombocytopenia according to the type of malaria. The relative risk for anemia and thrombocytopenia in malaria-affected individuals were calculated.

Results

Out of 824 patients, 616 (75%) were male and 208 (25%) were female with ages ranging from 18 to 55 years (33.2 ± 8.3). All patients included in the study had active malaria as determined by the presence ofschizont and ring stages of malarial parasites. On the fifth day after treatment with artemether and lumifenterine 80/480 mg, 768 (93%) patients were rendered malaria free and the remaining 56 (7%) patients were malaria free on the seventh day. The hematological parameters noted were hemoglobin, platelets count, and total leukocytes as well as differential counts (Table 1). Anemia and thrombocytopenia were present in 71% and 87% of the population, respectively. Clinical features are shown in Table 2 with four features significantly correlating ($p < 0.05$) with malaria, *i.e.*, fever, rigors/chills, headaches, and anemia. Species specific parameters are shown in Table 3, with anemia correlating positively with *Plasmodium falciparum* anemia ($p = 0.001$), while moderate and severe thrombocytopenia correlate well with *Plasmodium vivax* malaria ($p < 0.001$). Species specific statistical parameters such as prevalence, sensitivity, specificity, and positive and negative predictive values are shown in Table 4. Anemia in *falciparum* malaria was both sensitive and specific (80% and 100%) with a 100% positive predictive value, and anemia in *vivax* malaria was rather specific (80%) but had only 19% positive predictive value and 80% negative predictive value. Anemia in mixed malaria was neither sensitive nor specific. The specificity for severe thrombocytopenia in *vivax* and mixed malaria was 56% and 60%, respectively. Table 5 shows the relative risks of obtaining a particular hematological parameter in a malaria-affected cohort. The relative risk for people with malaria to contract anemia was 3.18. The relative risk of contracting moderate and severe thrombocytopenia in a cohort was 0.99 and 0.44 respectively.

Discussion

Hematological delinquencies are the hallmark of malaria, especially pronounced in *P. falciparum*, due to high parasite index [11]. The hematological changes previously reported in the literature include anemia, thrombocytopenia, atypical lymphocytosis, leucopenia, leukocytosis, neutropenia, neutrophilia, eosinophilia, monocytosis, and rarely DIC [10].

Clinical signs which statistically support a diagnosis of malaria include fever, headache, anemia, and rigors/chills, as reported in other studies [12,13].

Table 1. Hematological parameters in patients

S. No.	Hematological parameters	Reference ranges	Classification	Frequency (percentages)
1-	Hemoglobin	11-16 mg/dl	Anemia	584 (71%)
			Normal	240 (29%)
2-	Platelets	150,000-400,000 IU/L	Normal	108 (13%)
			Thrombocytopenia[$]	716 (87%)[$]
			a- Mild (<150,000 TO >50,000/L)	188 (26%)
			b- Moderate (<50,000 TO >20,000/L)	372 (52%)
			c- Severe (<20,000/L)	156 (22%)
3-	Leukocytes	4,000-11,000 IU/L	Normal	652 (79%)
			Leucopenia	148 (18%)
			Leukocytosis	24 (3%)
4-	Neutrophils	40-75%	Normal	768 (93%)
			Neutropenia	40 (5%)
			Neutrophilia	16 (2%)
5-	Lymphocytes	20-45%	Normal	700 (85%)
			Lymphopenia	0 (0%)
			Lymphocytosis	124 (15%)
6-	Eosinophil	1-6%	Normal	660 (80%)
			Eosinophilia	164 (20%)
7-	Monocytes	2-10%	Normal	800 (97%)
			Monocytosis	24 (3%)
8-	Basophil	0-1%	Normal	824 (100%)

S. No. = Serial number
n = 824
[$] The frequencies and percentages for each subcategory of thrombocytopenia were calculated out of 716 patients

We observed a myriad of hematological aberrancies ranging from simple anemia to severe life-threatening thrombocytopenia.

Anemia was found to be statistically significant in *P. falciparum* malaria patients in our study with a 100% positive predictive value (PPV), while PPV was only 19% for *vivax* malaria and 24% for mixed malaria. Anemia in *vivax* malaria was not sensitive but it was specific; while it was neither a sensitive nor a specific parameter in mixed malaria, we suggest that anemia is a more confounding parameter in *vivax*-affected patients and it more strongly rules in *falciparum* patients if found. Anemia in all 71% patients was hypochromic microcytic anemia, conforming to the findings of prior studies [11,14], but we also suggest that the RBC volume depends on the nutritional status of the patient and concomitant thalassemia, which is prevalent in our region. Anemia in malaria has been hypothesized as a result of hemolysis, peripheral removal of both parasitized and non-parasitized cells, and ineffective erythropoesis. Abnormally high levels of tumor necrosis factor have been implicated in the ineffective erythropoiesis [15].

There are conflicting reports regarding changes in white cell indices. In our study there was no dramatic decrease in the white blood indices, which supports the findings of Layla *et al*. [10] while contradicting others [11]. The differential counts in our study were all within the normal ranges. The occurrence of lymphocytosis in our cohort almost matched the findings of other studies [10]; however, none of our patients had lymphopenia.

Table 2. Clinical features in malaria patients

S. No.	Features	*P. falciparum* n (%)[#]	*P. vivax* n (%)[#]	Mixed malaria n (%)[#]	p-value
1-	Number of patients[$]	**472**	**160**	**192**	-
2-	Fever	472 (100%)	160 (100%)	192 (100%)	0.010[*]
3-	Chills/rigors	452 (96%)	132 (83%)	176 (92%)	0.016[*]
4-	Head aches	460 (97%)	160 (100%)	170 (89%)	0.035[*]
5-	Sweating	376 (80%)	120 (75%)	160 (83%)	0.331
6-	Anemia	472 (100%)	28 (18%)	92 (48%)	0.025[*]
7-	Myalgias	288 (61%)	0 (0%)	192 (100%)	0.198
8-	Vomiting	160 (34%)	48 (30%)	104 (54%)	1.00
9-	Jaundice	176 (37%)	32 (20%)	96 (50%)	0.98
10-	Splenomegaly	124 (26%)	20 (12.5%)	160 (83%)	0.311
11-	Abdominal pain	184 (39%)	32 (20%)	24 (12.5%)	0.766
12-	dizziness	96 (20%)	76 (47.5%)	60 (31.25%)	0.12
13-	Hepatomegaly	100 (21%)	32 (20%)	96 (50%)	0.843
14-	Diarrhea	88 (19%)	0 (0%)	0 (0%)	0.561
15-	Lymphadenopathy	12 (3%)	0 (0%)	41 (21.35%)	0.66

S. No. = Serial number
n = 824
[#] The percentages were calculated based on total number of patients in each group all percentages were round off to the nearest value.

Table 3. Species specific hematological parameters

S. No.	Parameter	Plasmodium Falciparum	Plasmodium Vivax	Mixed Malaria
1-	**Anemia (n = 584)**	472 (p = 0.001)[*]	110 (p = 1)	2 (p = 0.24)
	No Anemia (n = 240)	0	48 (p = NS)	192 (p = NS)
2-	**No Thrombocytopenia (n = 108)**	0	0	108 (p = 0.43)
	Mild Thrombocytopenia (n = 188)	188 (p = 0.511)	0	0
	Moderate Thrombocytopenia (n = 372)	284 (p = 0.34)	88 (p<0.001)[*]	0
	Severe Thrombocytopenia (n = 156)	2 (p = NS)	70 (P<0.001)[*]	84 (p = NS)

S. No. = Serial number
*p value significant
NS= Non-significant

Table 4. Prevalence, Sensitivity, Specificity and Predictive values of parameter detection in particular type of malaria[#]

S. No.	Parameter	Prevalence (95% C.I.)	Sensitivity (95% C.I.)	Specificity (95% C.I.)	PPV (95% C.I.)	NPV (95% C.I.)
1-	Anemia in *P. falciparum*	71 (68−74)	81 (77−84)	100 (98−100)	100 (98−100)	68 (62−72)
2-	Anemia in *P. vivax*	71 (68−74)	20 (16−22)	80 (74−84)	19 (17−22)	80 (77−83)
3-	Anemia in mixed malaria	71 (68−74)	0.3 (0−1.3)	20 (15−26)	24 (21−27)	76 (73−79)
4-	Mild Thrombocytopenia (*falciparum*)	87 (84−89)	26 (23−29)	0 (0−4)	36 (33−39)	64 (60−67)
5-	Moderate Thrombocytopenia (*falciparum*)	76 (73−79)	45 (41−49)	55 (48−62)	45 (42−49)	55 (51−58)
6-	Moderate Thrombocytopenia (*vivax*)	52 (49−56)	20 (17−25)	27 (23−32)	45 (42−49)	55 (51−58)
7-	Severe Thrombocytopenia (*falciparum*)	68 (65−71)	0.3 (0.0−1.4)	41 (35−47)	19 (16−21)	81 (78−84)
8-	Severe Thrombocytopenia (*vivax*)	76 (73−79)	11 (8−14)	56 (48−63)	19 (16−21)	81 (78−84)
8-	Severe Thrombocytopenia (Mixed)	78 (75−81)	13 (11−16)	60 (52−67)	19 (16−21)	0.81 (78−84)

S. No. = Serial number

The values are in percentages.

Reticuloendothelial system hyperplasia had been defined by previous authors in the form of hepatosplenomegaly and monocytosis [16,17]. In our study, monocytosis was present in only 3% of the patients, which further statistically supports the insignificance of hepatomegaly (0.843) and splenomegaly (0.311). Eosinophilia was present in 20% of the patients, which cannot exclusively be attributed to malaria as we did not rule out other parasitic infections prevalent in our area. To the best of our knowledge there does not appear to be any literature relating to changes in basophil counts.

Platelet abnormalities in malaria are both qualitative and quantitative. Low platelet count emerged as the strongest predictor of malaria, a previous observation which we confirmed [9]. Findings of thrombocytopenia with anemia is an important clue to the diagnosis of malaria in patients of acute febrile illness and this is in agreement with the findings of studies from Africa[9,18]. In the current study, 87% of the patients suffering from malaria showed some degree of thrombocytopenia. These figures are comparable to the observations of previous studies, in which the authors found thrombocytopenia in 59% to 71% of the patients [6]. Thrombocytopenia has been consistently attributed to *P. falciparum* malaria but is infrequently described with *vivax*. In our study we found moderate and severe thrombocytopenia to be statistically significant among *P. vivax* malaria patients and this observation supports previous reports in isolated literature pertaining to thrombocytopenia in *vivax* malaria [19,20,21]. Thrombocytopenia has been postulated to occur secondary to peripheral destruction, splenic sequestration, and platelet consumption secondary to DIC, but none of our patients showed any bleeding tendencies despite very low platelet counts. The presence of cellular bone marrow in some cases of malaria suggest that thrombocytopenia is not likely to be secondary to marrow failure; rather, the immune-medicated destruction of platelets has been postulated as the cause of thrombocytopenia [9]. Qualitative abnormalities of platelets are also found in malaria-infected patients. The most commonly observed abnormality is the formation of giant platelets which helps in releasing premature megakaryocytes secondary to thrombocytopenia [22].

In the peripheral blood of malaria-infected patients, low hemoglobin and low platelets are considered the most significant diagnostic parameters.

Table 5. Relative risk of anemia and thrombocytopenia in malaria cohort

S. No.	Parameter	RR (95% C.I.)
1-	No anemia	1.57 (1.38−1.78)
	Anemia	3.18 (2.25−4.49)
2-	Moderate thrombocytopenia	0.99 (0.98−1.03)
	Severe thrombocytopenia	0.44 (0.37−0.51)

S. No. = Serial number

Maina *et al*. have described that these parameters have sensitivity and specificity of 80% and 84%, respectively [9]. In our study, we observed anemia in *falciparum* malaria to be 80% sensitive and 100% specific, and thrombocytopenia of any severity was neither sensitive nor specific (maximum value of specificity 55%). Hence patients with acute febrile illness without localizing signs and having a combination of anemia and thrombocytopenia should be treated by the attending physician with a high suspicion of malaria infection, which can be confirmed with specific tests [9].

Conclusion

As malaria has a varied presentation profile, one should always keep in mind that investigating complete blood indices would increase the probability of detecting malaria in patients with acute febrile illness but will not serve as a specific diagnostic tool because these alterations can be encountered with other tropical diseases, such as dengue and typhoid. In malaria endemic regions, any patient with acute febrile illness showing the clinical triad of headache, fever, and rigors/chills, along with low platelet counts as well as anemia, irrespective of a malaria smear, should be thoroughly investigated for malaria.

References

1. WHO (1996) World Health Director General's Report. Fighting disease, fostering development. Geneva: WHO, 1996. Available at http://www.who.int/whr/1996/en/index.html. Last accessed 28 November 2012.
2. Agravat AH and Dhruva GA (2010) Hematological changes in patients of malaria. Journal of Cell and Tissue Research 10: 2325-2329.
3. Lathia TB, Joshi R (2004) Can hematological parameters discriminate malaria from non malarious acute febrile illness in the tropics? Indian J Med Sci 58: 239-244.
4. WHO. (1996) World Health Director General's report. Fighting disease, fostering development Geneva: WHO, 1996.
5. Kondrachine AV, Trigg PI (1997) Global overview of malaria. Indian J Med Res 106: 39-52.
6. Memon AR and Afsar S (2006) Thrombocytopenia in hospitalized malaria patients. Pak J Med Sci 22: 141-143.
7. Barnish G, Bates I, Iboro J (2004) Newer drug combinations for malaria. BMJ 328: 1511-1512.
8. .WHO (2000) New perspectives, malaria diagnosis. Geneva, 2000.
9. Maira RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, Jones D, Ogutu BR (2010) Impact of *Plasmodium falciparum* infection on hematological parameters in children living in western Kenya. Malaria J 9: 1-11.
10. Bashawri LAM, Mandil AA, Bahnassy AA, Ahmed MA (2002) Malaria: hematological aspects. Annals of Saudi Medicine 22: 372-377.
11. Facer CA (1994) Hematological aspects of malaria. In: Infection and Hematology. Oxford: Butterworth Heinmann Ltd. 259-294.
12. Luxemburger C, Nosten F, Kyle DE, Kiricharoen L, Chongsuphajaisiddhi T, White NJ (1998) Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission. Trans R Soc Trop Med Hyg 92: 45-49.
13. Seidi MGM, El Tahir A, Mohammed AS (1998) Clinical aspects of malaria in the Asir region, Saudi Arabia**.** Ann. Saudi Med 18:15-17.
14. Beales PF (1997) Anemia in malaria control: a practical approach. Ann Trop Med Parasitol91:713-8.
15. Wickramasinghe SN, Abdalla SH (2000) Blood and bone marrow changes in malaria. In Bailliere's Clin Hematol. Vol 13. Harcourt Pub Ltd. 277-299.
16. Abdalla SH (2004) Leukocytes in malaria. In Abdalla SH and Pasvol G, editors. Malaria: a hematological perspective. Vol 4. London: Imperial College press 129-168.
17. Chiwakata CB, Hammer CJ, Dietrich M (2000) High levels of inducible nitric oxide synthase mRNA are associated with increased monocyte count in blood and have beneficial role in *P. falciparum* malaria. Infect Immun 68: 394-99.
18. Adedapo AD, Falade CO, Kotila RT, Ademowo GO (2007) Age as a risk factor for thrombocytopenia and anemia in children treated for acute uncomplicated *falciparum* malaria. J Vector Borne Dis 44: 266-271.
19. Makkar RP, Mukhopadhyay S, Monga A, Monga A, Gupta AK (2002) *Plasmodium vivax* malaria presenting with severe thrombocytopenia. Braz J Infect Dis 6: 263-265.
20. Lacerda MV, Alexandre MA, Santos PD, Arcanjo AR, Alecrim WD, Alecrim MG (2004) Idiopathic thrombocytopenic purpura due to vivax malaria in the Brazilian Amazon. Acta Trop 90: 187-190.
21. Rodriguez-Morales AJ, Sanchez E, Vargas M, Piccolo C, Colina R, Arria M, Franco-Paredes C (2005) Occurrence of thrombocytopenia in *Plasmodium vivax* malaria. Clin Infect Dis 41: 130.

22. Ladhani S, Lowe B, Cole AO,Kowuondo K,Newton CR (2002) Change in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. Br J Hematol 119: 839-847.

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