ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

www.jhas-bsh.com

Journal of Hematology and Allied Sciences


*Original Research*

# Profile of hematological parameters in plasmodium falciparum malaria: A study from West Bengal

Swapan Kumar Sinha[1], Debes Pal[1], Malini Garg[2]

[1]Pathology, Jagannath nGupta Institute of Medical Sciences & Hospital (JIMSH), Budge Budge, Kolkata-700137, West Bengal-India, [2]Hematology, Nil Ratan Sircar Medical college and hospital, 138, AJC Bose Road, Kolkata-700014, West Bengal-India.

***Corresponding author:**
Malini Garg
Hematology, Nil Ratan Sircar Medical college and hospital, 138, AJC Bose Road, Kolkata-700014, West Bengal-India.

malinigarg13@gmail.com

Received : 13-March-2021
Accepted : 02-May-2021
Published : 31-May-2021

**DOI**
10.25259/JHAS_4_2021

**Quick Response Code:**

## ABSTRACT

**Objectives:** Anemia is one of the main clinical presentations of severe malaria caused by *P. falciparum* and one of the major morbidities of malaria. This study was undertaken to explore the burden of anemia and hematological derangement in patients with *P. falciparum* malaria.

**Material and Methods:** A cross sectional study was conducted on 186 patients of all age groups suffering from falciparum malaria. Complete blood count (CBC) with peripheral blood smear, reticulocyte count, liver function test (LFT) and plasma hemoglobin were done in all patients. Direct Coombs test (DCT) and urine for hemoglobin was estimated in 19 patients where hemolytic anemia was suspected.

**Results:** Anemia was seen in majority (78.7%) of the patients; 82.7% of males, 70.6% of females and 87.2% of children had anemia. Thrombocytopenia was seen in 9.1% cases. Unconjugated hyperbilirubinemia was seen in 33.8% patients though plasma hemoglobin was raised in only 1.1% patients. DCT was positive in 3 patients (15.7%) and hemoglobinuria was seen in 2 patients (10.5%).

**Conclusion:** The present study revealed that anemia is one of the common manifestations of falciparum malaria and requires special attention to reduce the burden of this morbidity.

**Keywords:** Falciparum malaria, Hematological changes, Anemia, Morbidity

## INTRODUCTION

*Plasmodium falciparum*, a protozoan parasite causes the most virulent form of human malaria and is responsible for one of the major cause of increased morbidity and mortality of children globally, especially in developing countries.[1] The parasite in the asexual blood stage that infects the mature red blood cell (RBC) causes all of the symptoms and malarial associated pathologies. Uncomplicated malaria is commonly associated with cyclical fevers and chills and its periodicity reflects the intraerythrocytic cycle. Multiple additional pathologies are seen in severe malaria that includes cerebral malaria (that results from adhesion of infected erythrocytes to the endothelium of the brain), lactic acidosis, and anemia.[1,2] Of these, the least understood is anemia. Although anemia contributes to one of the major health problems for children and pregnant women in endemic areas and a main cause of the infant mortality associated with malaria, there are very limited studies from this geographical region addressing this issue. There are multiple pathologies that complicate malaria in anemia, in which destruction of infected and uninfected erythrocytes contribute the most, also inadequate response to anemia also plays a role. *Plasmodium vivax,* which is largely a non-lethal malaria, also causes anemia that may be severe.[3]

Malarial anemia is a multifactorial disease; the complex etiological basis for anemia in malaria is only partially understood. Severe anemia is one of the main clinical presentations of severe malaria caused by *P. falciparum*[4] and one of the major morbidities associated with malaria. The etiology of severe anemia associated with malaria in endemic areas may include a number of discrete as well as overlapping features, the main being lysis of infected and uninfected RBCs,[5] splenic sequestration of RBCs,[6] dyserythropoiesis, and bone marrow suppression.[7]

The insight into the prevalence of anemia associated with malaria in children as well as adults will provide valuable information to the healthcare providers. This will enable the appropriate allocation of scarce resources for prevention, proper health management, and control of these morbidities. The present study was done with the aim to explore the burden of anemia and hematological derangements in patients with falciparum malaria and to evaluate the attributable risk of anemia caused by malaria.

## MATERIAL AND METHODS

A cross sectional study was conducted on 186 patients of falciparum malaria, over a period of 2 years. Complete hemogram was done using Sysmex XP100 autoanalyser (Sysmex corp., US), along with peripheral blood smear examination and strip tests (HRP2 strip tests) for malaria in all patients. The cut of anemia was taken according to standard anemia definition by World Health Organization. Platelet count < $100 \times 10^9$/L, was taken as thrombocytopenia [Table 1]. Corrected reticulocyte count (CRC) was performed using supravital staining and plasma hemoglobin were also performed using automated analyzer in all patients. Liver function test was performed in all patients using XL-600 Erba Mannheim Autoanalyser (Erba Mannheim, London, UK). Direct Coombs test (DCT) was done in 19 patients with suspected hemolytic anemia using gel card technique. Urine for hemoglobin was tested using strip tests in 19 patients with suspected hemolytic anemia. Descriptive analysis of the data was done.

## RESULTS

The present study included 186 patients suffering from falciparum malaria. Majority of the patients (74.7%; $n = 139$) were in the age group of 15–60 years with the rest (25.2%; $n = 47$) being below 15 years. Male-to-female ratio was 1.39 : 1. Anemia was seen in majority of the patients (78.7%), with 82.7% of males, 70.6% of females and 87.2% of children being anemic [Table 1]. Reticulocyte count was less than 1.5% in 32.8% patients, with thrombocytopenia (platelet count < $100 \times 10^9$/L) in 9.1%. Plasma hemoglobin was raised (>100 mg/L) in only 1.1%, patients whereas unconjugated hyperbilirubinemia was seen in 33.8% (63/186) patients. Hemolysis was suspected in 19 patients DCT and urine for hemoglobin was tested in those patients. DCT was positive in 3 patients (15.7%) and hemoglobinuria was seen in 2 patients (10.5%). Out of all the patients with anemia ($n = 149$), 42.3% patients had hemolytic anemia (63/149), 2.01% had autoimmune hemolytic anemia (3/149) and for the rest, the cause of anemia was not known. The results are shown in Table 1.

## DISCUSSION

In this present study, majority of the patients with falciparum malaria had anemia (78.7%) and 9.1% patients had low platelet count.

A study by Sumbele *et al.*[8] from a different geographical region had shown that prevalence of anemia was significantly higher in children who were positive for malaria parasite

**Table 1:** Laboratory findings in patients with P. falciparum malaria ($n = 186$).

| Parameters | Patients evaluated | Number (cases) | Percentage (cases) |
|---|---|---|---|
| Anemia ($n = 186$) | Adults | | |
| | Male ($n = 81$) (Hb <13 g/dl) | 67 | 82.7 |
| | Female ($n = 58$) (Hb < 12 g/dl) | 41 | 70.6 |
| | Children ($n = 47$) (Hb < 12 g/dl) | 41 | 87.2 |
| Reticulocyte count (>1.5%) ($n = 186$) | | 61 | 32.8 |
| Platelet counts (< $100 \times 10^9$/L) ($n = 186$) | | 17 | 9.1 |
| Plasma or serum free hemoglobin (>100 mg/L) ($n = 186$) | | 2 | 1.1 |
| DCT ($n = 19$) | | 3 | 15.7 |
| Unconjugated hyperbilirubinemia (>1.5 mg/dl) ($n = 186$) | Adults | | |
| | Male ($n = 81$) | 23 | 28.3 |
| | Female ($n = 58$) | 21 | 36.2 |
| | Children ($n = 47$) | 19 | 40.4 |
| Hemoglobinuria ($n = 19$) | | 2 | 10.5 |

and those with fever, in comparison to their respective counterparts. In their study, moderate-to-severe anemia and moderate-to-severe malarial anemia was detected in 38.0% and 15.3% of the participants, respectively. Also, lower mean white blood cell, lymphocyte, and platelet counts were seen in children with moderate-to-severe malarial anemia, while the mean granulocyte count was significantly higher in these children.

As shown by various previous studies, the pathogenesis of malarial anemia is multifactorial attributing to multiple causes.[9-13] As, malarial parasite is an intraerythrocytic parasite, schizont rupture causes obligatory destruction of these infected red cells. However, the accelerated destruction of non-parasitized red cells that parallels disease severity is also an important contributor to anemia.[9] As documented in previous studies, it has been shown that loss of unparasitized erythrocytes mainly account for approximately 90% of anemia resulting from a single infection.[9,14-16] Parasitemia in falciparum malaria commonly exceeds 1% (of red cells parasitized), and in severe disease may exceed even 10%. Severe falciparum malaria is characterized by heavy parasite burden and thus there is rapid development of anemia in these patients. Hemolysis of unparasitized red cells is the main contributor to this usually rapid decline in hematocrit.[9,14-16]

As seen in the present study, reticulocyte count was increased in 32.8% patients. Plasma or serum free hemoglobin was raised in 1.1% patients, with unconjugated hyperbilirubinemia seen in 33.8% and hemoglobinuria seen in only 2 of the tested 19 patients, suggesting that these patients also had on going hemolysis.

As shown by various authors, apart from the reason discussed above, hemolytic anemia seen in malaria is also attributed to bone marrow dyserythropoiesis during and immediately after the acute illness.[17,18] Dyserythropoiesis seen in malaria is mainly thought to be related to intramedullary production of mediators (proinflammatory cytokines, nitric oxide, lipo-peroxides, bioactive aldehydes) causing suppression of erythropoiesis and also, in some previous studies, these mediators have been shown to be involved in causing red cell precursor apoptosis.[19,20] In severe malaria caused by P. falciparum, it has been shown that the entire red cell population becomes less deformable.[21,22] Although, the mechanisms that could be responsible for reduced deformability of uninfected erythrocyte have not been identified clearly, nevertheless, there is an evidence that increased oxidative damage in acute malaria might compromise red cell membrane function and thus leads to reduced deformability.[22-24]

The role of RBC membrane bound antibody, i.e. Coombs'-positive hemolysis, in malarial anemia is poorly understood. This present study shows only 3 patients had positive DCT. There are a few case reports and studies demonstrating positive coombs test in patients with malaria.[25,26] In one of the very old studies on 131 soldiers evacuated from Vietnam with drug resistant *Plasmodium falciparum* malaria, 4 patients were found with a positive DAT of IgG type.[25]

Some previous studies have shown increased RBC immunoglobulin binding in malaria, whereas others have not.[27,28] As malaria is associated with lowered clearance threshold for splenic RBC removal, increased antibody or complement binding detections becomes a challenging task. Nevertheless, studies in Kenyan children with severe malarial anemia have shown increased surface IgG and immune complexes and also deficiencies in the complement regulatory proteins CR1 and CD55 have been detected in these patients.[29,30] In the same study, it was also seen that the circulating erythrocytes in these children were more susceptible to phagocytosis in comparison to those of controls.[30]

As suggested by a few authors, anemia in these patients can also be attributed to splenomegaly. In acute malaria, it is seen that the spleen reorganizes and enlarges rapidly, resulting in increased clearance capacity and lowered splenic threshold for the clearance of abnormal erythrocytes, either because of antibody coating or reduced deformability.[31,32]

And the last but not the least, anemia in patients can be due to nutritional causes also. Iron deficiency is one of the major cause of nutritional deficiency anemia and also very common in malaria endemic areas. Iron deficiency not only causes anemia, but in young children iron deficiency is associated with neurodevelopmental delay also. Malaria does not cause iron deficiency perse, but the two causes can go hand in hand in the same patient. Though, it has been shown that iron deficiency does reduce the incidence of severe malaria due to multiple reasons.[33] Nevertheless, iron deficiency and malaria can still often coincide in the same patient. In the present study, parameters of iron deficiency anemia were not evaluated, however iron deficiency being so common in India, it must be kept in mind while evaluating patients for anemia.

**Limitations of the study**

Causes of nutritional deficiency were not ruled out in the present study. Further studies with more number of sample size, with more tests like serum haptoglobin levels and lactic acid dehydrogenase (LDH), and showing impact of malaria treatment on anemia may make the picture of this morbidity more clear. Also further studies including follow up of these patients can further enhance the knowledge regarding the same.

## CONCLUSION

As, most of the patients in the present study had anemia and few having features of hemolytic anemia with 3 patients even DCT positive, with the present study we conclude that anemia is one of the common manifestations of falciparum malaria. With this study, we suggest, that appropriate allocation of the available resources for control of anemia may reduce the burden of this morbidity. Further studies are needed for the same.

### Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, *et al.* Malaria and the red cell. Hematology Am Soc Hematol Educ Program 2002:35–57.
2. Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, Roberts DJ. Malarial anemia of mice and men. Blood 2007;110:18–28.
3. Ekvall H. Malaria and anemia. Curr Opin Hematol 2003;10:108–14.
4. Achidi EA, Apinjoh TO, Anchang-Kimbi JK, Mugri RN, Ngwai AN, Yafi CN. Severe and uncomplicated falciparum malaria in children from three regions and three ethnic groups in Cameroon: prospective study. Malaria J 2012;11:1–2.
5. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, terKuile FE, Chongsuphajaisiddhi T, White NJ. Factors contributing to anemia after uncomplicated falciparum malaria. Am J Trop Med Hyg 2001;65:614–22.
6. Buffet PA, Safeukui I, Milon G, Mercereau-Puijalon O, David PH. Retention of erythrocytes in the spleen: a double-edged process in human malaria. Curr Opin Hematol 2009;16:157–64.
7. Phillips RE, Looareesuwan S, Warrell DA, Lee SH, Karbwang J, Warrell MJ, *et al*. The importance of anemia in cerebral and uncomplicated falciparum malaria: role of complications, dyserythropoiesis and iron sequestration. Q J Med 1986;58: 305–23.
8. Sumbele IU, Sama SO, Kimbi HK, Taiwe GS. Malaria, moderate to severe anemia, and malarial anemia in children at presentation to hospital in the Mount Cameroon area: a cross-sectional study. Anemia 2016;2016.
9. Looareesuwan S, Davis TME, Pukrittayakamee S, Supanaranond W, Desakorn V, Silamut K, *et al.* Erythrocyte survival in severe falciparum malaria. Acta Trop 1991;48:263–70.
10. Burgmann H, Looareesuwan S, Kapiotis S, Viravan C, Vanijanonta S, Hollenstein U, *et al.* Serum levels of erythropoietin in acute Plasmodium falciparum malaria. Am J Trop Med Hyg 1996;54:280–3.
11. Vedovato M, De Paoli Vitali E, Dapporto M, Salvatorelli G. Defective erythropoietin production in the anemia of malaria. Nephrol Dial Transplant 1999;14:1043–4.
12. Price RN, Simpson J, Nosten F, Luxemburger C, Hkirjaroen L, terKuile F, *et al.* Factors contributing to anemia in uncomplicated falciparum malaria. Am J Trop Med Hyg 2001;65:614–22.
13. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, *et al.* The anemia of plasmodium vivax malaria. Malar J 2012;11:135.
14. Looareesuwan S, Merry AH, Phillips RE, Pleehachinda R, Wattanagoon Y, Ho M, *et al.* Reduced erythrocyte survival following clearance of malarial parasitaemia in Thai patients. Br J Haematol 1987;67:473–8.
15. Collins WE, Jeffery GM, Roberts JM. A retrospective examination of anemia during infection of humans with Plasmodium vivax. Am J Trop Med Hyg 2003;68:410–2.
16. Jakeman GN, Saul A, Hogarth WL, Collins WE. Anemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. Parasitology 1999;119:127–33.
17. Casals-Pascual C, Kai O, Cheung JO, Williams S, Lowe B, Nyanoti M, *et al.* Suppression of erythropoiesis in malarial anemia is associated with hemozoin in vitro and in vivo. Blood 2006;108:2569–77.
18. Aguilar R, Moraleda C, Achtman AH, Mayor A, Quintó L, Cisteró P, *et al.* Severity of anemia is associated with bone marrow haemozoin in children exposed to Plasmodium falciparum. Br J Haematol 2014;164:877–87.
19. Lamikanra AA, Theron M, Kooij TW, Roberts DJ. Hemozoin (malarial pigment) directly promotes apoptosis of erythroid precursors. PLoS ONE 2009;4:e8446.
20. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, Ong'echa JM. Severe malarial anemia: innate immunity and pathogenesis. Int J Biol Sci 2011;7:1427–42.
21. Dondorp AM, Angus BJ, Chotivanich K, Silamut K, Ruangveerayuth R, Hardeman MR, *et al.* Red blood cell deformability as a predictor of anemia in severe falciparum malaria. Am J Trop Med Hyg 1999;60:733–7.
22. Griffiths MJ, Ndungu F, Baird KL, Muller DP, Marsh K, Newton CR. Oxidative stress and erythrocyte damage in Kenyan children with severe Plasmodium falciparum malaria. Br J Haematol 2001;113:486–91.
23. Nuchsongsin F, Chotivanich K, Charunwatthana P, Omodeo-Salè F, Taramelli D, Day NP, *et al.* Effects of malaria heme products on red blood cell deformability. Am J Trop Med Hyg 2007;77:617–22.
24. Matthews K, Duffy SP, Myrand-Lapierre ME, Ang RR, Li L, Scott MD, *et al.* Microfluidic analysis of red blood cell deformability as a means to assess hemin-induced oxidative stress resulting from Plasmodium falciparum intraerythrocytic parasitism. Integr Biol (Camb) 2017;9:519–28.

25. Adner MM, Altstatt LB, Conrad ME. Coombs'-positive hemolytic disease in malaria. Annals Intern Med 1968; 68:33–8.
26. Abdalla S, Weatherall DJ. The direct antiglobulin test in P. falciparum malaria. Br J Haematol 1982;51:415–25.
27. Facer CA. Direct Coombs' antiglobulin reactions in Gambian children with Plasmodium falciparum malaria. II. Specificity of erythrocyte bound IgG. Clin Exp Immunol 1980;39:279–88.
28. Merry AH, Looareesuwan S, Phillips RE, Chanthavanich P, Supanaranond W, Warrell DA, *et al.* Evidence against immune haemolysis in falciparum malaria in Thailand. Br J Haematol 1986;64:187–94.
29. Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. Red cell surface changes and erythrophagocytosis in children with severe Plasmodium falciparum anemia. Blood 2000;95:1481–6.
30. Stoute JA, Odindo AO, Owuor BO, Mibei EK, Opollo MO, Waitumbi JN. Loss of red blood cell-complement regulatory proteins and increased levels of circulating immune complexes are associated with severe malarial anemia. J Infect Dis 2003;187:522–5.
31. Lee SH, Looareesuwan S, Wattanagoon Y, Ho M, Wuthiekanun V, Vilaiwanna N, *et al.* Antibody-dependent red cell removal during P. falciparum malaria: the clearance of red cells sensitized with an IgG anti-D. Br J Haematol 1989; 73:396–402.
32. Ho M, White NJ, Looareesuwan S, Wattanagoon Y, Lee SH, Walport MJ, *et al.* Splenic Fc receptor function in host defense and anemia in acute Plasmodium falciparum malaria. J Infect Dis 1990;161:555–61.
33. Gwamaka M, Kurtis JD, Sorensen BE, Holte S, Morrison R, Mutabingwa TK, *et al.* Iron deficiency protects against severe Plasmodium falciparum malaria and death in young children. Clin Infect Dis 2012;54:1137–44.

**How to cite this article:** Sinha SK, Pal D, Garg M. Profile of hematological parameters in plasmodium falciparum malaria: A study from West Bengal. J Hematol Allied Sci 2021;1(1):28–32.