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Original Research Article

Study of variations in plasma haemoglobin levels in relation to species, type and severity of parasitaemia in malaria patients

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Abstract

Measurement of plasma haemoglobin is useful in variety of clinical condition. Malaria is one such condition, resulting in haemolytic anaemia thereby altering plasma haemoglobin levels. In malaria, intravascular haemolysis causes the haemoglobin-mediated vasculopathy and end-organ toxicity. This study was done to find the extent of intravascular haemolysis in malarial patients by detecting the plasma haemoglobin levels and correlating the extent of haemolysis with different species of malarial parasites. 50 cases of malaria diagnosed by peripheral smear at the central diagnostic laboratory in A.J. Institute of Medical Sciences, Mangalore, India, were subjected to plasma haemoglobin estimation by benzidine method. Plasma haemoglobin was increased in 3 out of 4 cases in Plasmodium falciparum 3+ cases. One case of Plasmodim falciparum 2+ and Plasmodium vivax 3+ showed increase in the plasma haemoglobin levels. The design and testing of haemoglobin-binding and haemoglobin metabolism therapeutics should be considered for malaria especially Plasmodium falciparum malaria to prevent complications such as haemoglobin mediated vasculopathy and end organ damage.

Keywords: Haemolysis; Malaria; Plasma haemoglobin; Parasitaemia

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1. Introduction

Measurement of plasma haemoglobin is useful in variety of clinical conditions. Malaria is one such condition, resulting in haemolytic anaemia thereby altering plasma haemoglobin levels. Premature destruction of red cells in hemolytic anemia may occur by two mechanisms. Firstly, the red cells undergo lysis in the circulation and release their contents in plasma (intravascular haemolysis). In these cases the plasma haemoglobin rises substantially and part of it may be excreted in the urine (Haemoglobinuria). Plasma haemoglobin estimation detects intravascular haemolysis. Secondly, the red cells are taken by cells of the reticuloendothelial system where they are destroyed and digested (extravascular haemolysis). In extravascular haemolysis, plasma haemoglobin is therefore, unaffected. Intravascular haemolysis is caused

by mechanical injury, complement fixation, infection by intracellular parasites such as malaria or exogenous toxic factors. In India malaria is caused mainly by Plasmodium vivax and Plasmodium falciparum. In malaria, intravascular haemolysis causes the haemoglobin-mediated vasculopathy and end-organ toxicity. This study was done to find the extent of intravascular haemolysis in malarial patients by detecting the plasma haemoglobin levels and correlating the extent of haemolysis with different species of malarial parasites and degree of parasitaemia.

2. Materials and methods

Fifty cases of malaria diagnosed by peripheral smear at the central diagnostic laboratory in A.J. Institute of Medical Sciences, Mangalore, India, were included in the study. Cases were selected over a period of 1 year from March 2011 to February 2012. Peripheral smear was used for diagnosing the species and evaluating the degree of parasitaemia. Degree of parasitaemia was classified into 1+, 2+, and 3+, depending on the presence of number of ring forms or gametocytes in the peripheral smear. 1-5 (ring forms or gametocytes) per 10 fields on 100X was classified as 1+. 5 -10 (ring forms or gametocytes) per 10 fields on 100X were classified a 2+. More than 10 (ring forms or gametocytes) per 10 fields on 100X was classified as 3+. Plasma haemoglobin was estimated by using benzidine method. The normal plasma haemoglobin level is 10-40 mg/l.

3. Results

Thirty cases were positive for Plasmodium vivax (Pl v) alone. 12 cases were positive for Plasmodium falciparum (Pl f) alone. Eight cases were positive for both Plasmodium vivax and Plasmodium falciparum. Total cases were 58 because 8 cases showed co-infection with both Plasmodium vivax and Plasmodium falciparum. Out of total 38 cases of Plasmodium vivax, 11 cases were 1+, 22 cases were 2+, 5 cases were 3+. Out of 20 cases of Plasmodium falciparum, 12 cases were 1+, 4 cases were 2+ and 4 cases were 3+. Plasma haemoglobin ranged from 12 to 34.3 mg/l in 1+, 12 to 42 mg/l in 2+, 12 to 48 mg/l in 3+ infections with Plasmodium vivax. In Plasmodium falciparum, plasma haemoglobin ranged from 12 to 36 mg/l in 1+, 21 to 42 mg/l in 2+ and 36 to 71 mg/l in 3+. Plasma haemoglobin was increased in 3 out of 4 cases in Plasmodium falciparum 3+ cases. Plasma haemoglobin was increased in one case of Plasmodium vivax 3+ and one case of Plasmodium falciparum 2+.

4. Discussion

10-20% of normal erythrocyte destruction occurs intravascularly. Special features characterize those situations in which red cells are destroyed within circulation rather than within macrophages. When this happens, haemoglobin is discharged directly into the circulation from which it is removed by several mechanisms. At low rates of release of haemoglobin into plasma all of the haemoglobin is found to be attached to haptoglobin. This specific haemoglobin-binding protein was first detected in plasma by its ability to enhance the peroxidase activity of haemoglobin.[1] At low concentrations, plasma haemoglobin may be measured by means of the benzidine reaction.[2] which allows detection not only of haemoglobin but also of any other heme pigments that may be present. Particularly high values, upto 1000mg/dL, are found only in patients with disorders associated with predominantly intravascular haemolysis.[3] Malaria is an acute, chronic or recurrent febrile disease caused in humans by four species of plasmodia: P. vivax, P. falciparum, P. malariae, and p. ovale. Malaria is spread by mosquitoes of the genus Anopheles.

Anaemia is a common sign of malaria.[4] It is particularly characteristic of Plasmodium falciparum malaria because of greater extent of

Table 1: Plasma haemoglobin (Pl Hb) levels in mg/l in relation to species, type and severity of parasitaemia of Plasmodium vivax (Pl v) and Plasmodium falciparum (Pl f) infection.

Number of cases	Pl v 1+ Pl Hb	Pl v 2+ Pl Hb	Pl v 3+ Pl Hb	Pl v 1+ Pl Hb	Pl v 2+ Pl Hb	Pl v 3+ Pl Hb
1.	25.7	21	12	12	42	36
2.	18.5	32	36	12	25.7	48
3.	34.3	12.8	21	34	36	64
4.	17.4	24	32	25	21	71
5.	12	12	48	24	-	-
6.	12	17.4	-	12	-	-
7.	14.2	36	-	21	-	-
8.	34	12.8	-	18	-	-
9.	12.8	12	-	36	-	-
10.	25.7	24	-	10	-	-
11.	18.5	32	-	12.8	-	-
12.	-	25.7	-	21	-	-
13.	-	24	-	-	-	-
14.	-	18.5	-	-	-	-
15.	-	24	-	-	-	-
16.	-	29	-	-	-	-
17.	-	22	-	-	-	-
18.	-	36	-	-	-	-
19.	-	11	-	-	-	-
20.	-	18.5	-	-	-	-
21.	-	24	-	-	-	-
22.	-	36	-	-	-	-

red cell parasitazation with this species. Excessive destruction of red cells is the most important factor in the pathogenesis of anaemia.[4] Erythrocyte life span is shortened.[5] Haptoglobin disappears from the serum and osmotic fragility is increased. Haemoglobin digestion and cell disruption by the parasite are obvious causes of haemolysis.[6,7] Intravascular haemolysis is a pathological mechanism in several human diseases, including multiorgan dysfunction after either massive red blood cell transfusion or haemoglobin-based blood substitute therapy, the haemoglobinopathies, malaria, and other acquired and genetic hemolytic conditions.[8] Intravascular haemolysis releases haemoglobin and enzymes, such as arginase 1, into plasma. An efficient haemoglobin and heme scavenging system, consistenting of haptoglobin, CD163, and haemopexin, sequesters these redox-active molecules. When these scavenging systems are saturated by excess haemoglobin, cell free plasma haemoglobin accumulates and

extravasates within the vasculature and organs.[9] The haemoglobin-mediated vasculopathy and end-organ toxicity is likely relevant to haemolysis in malaria. The new studies of red blood cell storage[10-12] support a growing appreciation that intravascular haemolysis represents a fundamental mechanism for human disease.[13-15]

5. Conclusion

Plasma haemoglobin levels were increased in one case of Plasmodium falciparum infection with 2+ parasitaema and 3 cases of Plasmodium falciparum with 3+ parasitaemia. Plasma haemoglobin levels were increased in one case of Plasmodium vivax infection 3+ parasitaemia. The haemoglobin-mediated vasculopathy and end-organ toxicity is likely relevant to intravascular haemolysis in malaria. Therefore, plasma haemoglobin estimation should be considered in malaria especially falciparum malaria to detect intravascular haemolysis and to prevent the haemoglobin-mediated vasculopathy and end-organ toxicity.

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