Study of Methaemoglobin in Malaria Patients

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Abstract To estimate the concentration of methemoglobin (MetHb) in malaria patients and correlate with severity of malaria infection. This prospective study included 30 untreated cases of malaria confirmed by Quantitative Buffy Coat (QBC) test and 30 age sex matched non-malarial cases taken as controls. All the patients underwent thorough clinical examination and routine biochemical investigation. Methemoglobin levels were estimated by spectrophotometric (co-oxymeter) method on day 1 and day 10 of diagnosis of all study group patients and correlated with clinical profile and severity. Out of 30 malaria patients 22 were males and eight were females. The clinical presentations in complicated malaria group ($n = 21$) were fever 21 (100 %), anemia 17 (80.95 %), renal failure 12 (52.38 %) and coma/convulsion 5 (23.8 %). The mean age of the study group was 41.66 years. Mean MetHb in complicated malaria on day 1 was 2.55 ± 1.75 % and day 10 was 10.69 ± 8.19 % (statistically significant). The overall mortality was 13 (43.33 %) among study group while 5 (16.66 %) was found among control group. Mean MetHb who died ($n = 13$) on day 1 was 3.144 ± 1.829 % and ($n = 8$) on day 10 it was 19.982 ± 8.406 %. Increase in level of methaemoglobin is detrimental to the body and is associated with increase in mortality. Routine MetHb estimation may be used as a prognostic indicator in the management

of malaria patients. It is suggested that addition of drugs which reduce MetHb may be tried along with antimalarial drugs to decrease morbidity and mortality in malaria.

Keywords Methaemoglobin · Prognosticator · Malaria

Introduction

Malaria is a protozoan disease transmitted by the bite of infected anopheles mosquitoes. It is the most important of the parasitic disease of humans with transmission in 108 countries containing 3 billion people and causing nearly 1 million deaths each year. During malaria infection and after invading an erythrocyte the growing malarial parasite progressively consumes and degrades intracellular proteins principally hemoglobin. MetHb is generated by oxidation of haem iron moieties to ferric state causing characteristic brown muddy colour resembling cyanosis. MetHb is unable to bind oxygen leading to poor tissue oxygenation. In health MetHb should be ≤ 3 % of total Hb. MetHb causes cerebral ischemia at levels more than 15 % and becomes lethal if it is more than 60 %.

Methaemoglobinemia is a type of haemoglobinopathy. There are two types of methaemoglobinemia: congenital and acquired. Congenital methaemoglobinemia arise from globin gene mutation that stabilizes iron in ferric state (HbM Iwata) or from mutations that impairs the enzymes that reduce MetHb to haemoglobin (methaemoglobin reductase, NADP diaphorase) [1]. Acquired methaemoglobinemia occurs when RBCs are exposed to oxidizing agents producing MetHb. Implicated agents include phenacetin, lignocaine, notably nitrate and nitrite containing compounds [2–5]. It has also been documented in *P. vivax* malaria patients associated with high doses of

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primaquin treatment [6, 7]. In malaria infections, modification of haemoglobin molecules lead to acquired methaemoglobinemia. As the Indian study has been scarce in this regard, the present work has been undertaken with interest to estimate the concentration of MetHb with malaria patients and correlate with severity and prognosis of malaria infection.

Materials and Methods

The present study was carried out in department of medicine, V.S.S Medical College, Burla, a tertiary care referral centre from October 2009 to September 2011 in collaboration with Clinical Hematology Department of S.C.B MC Cuttack. Thirty untreated cases of malaria identified by positive QBC were included in the present study. Immunochromatographic test (ICT) was done in all the cases for diagnostic corroboration. Both thick and thin blood smear were examined in all the cases for accessing the degree of parasitemia as: <1 parasite/HPF = +, 1–10 parasites/HPF = ++, 11–100 parasites/HPF = +++, >100 parasites/HPF = +++++. Thirty cases (Afebrile, ischemic heart disease, stroke without malaria parasite infection) who were age and sex matched were included in the control group. Because of high prevalence of haemoglobinopathy in Odisha state, all cases (study and control group) were screened by Hb variant analysis (Biorad II) and any patient with any haemoglobinopathy were excluded from the study. The level of G6PD activity was estimated in all the cases (both study and control group) by dye reduction method and any patient having abnormal value was excluded from the study. The study was conducted according to the principles expressed in declaration of Helsinki and approved by the ethics committee of the institution. Written informed consent was obtained from patients or parents or guardians of all study group and control group patients. Co-existing febrile illness, enteric fever, urinary tract infection or pneumonia were excluded from the study. Detailed clinical history, physical examinations were done of all the study group patients. Laboratory investigations included Complete Blood Count (CBC) with reticulocyte count by 5 part differential hematology analyzer (Sysmex XT 2000iV), MP (microscopy, QBC or ICT), Blood sugar, serum urea and creatinine, serum sodium and potassium. Chest X-ray, ultrasound of abdomen and Liver Function Test (LFT) were done in all cases. Serum lactic dehydrogenase (LDH) was estimated by automated colorimetric method in all cases. The MetHb was estimated by spectrophotometric method (co-oxymetry) on day 1 and day 10 of diagnosis. The patients were grouped as complicated malaria (those having any one of the complications like cerebral malaria,

acidosis, renal failure, jaundice, convulsion, hypotension/shock, pulmonary edema) and uncomplicated malaria (absence of all these complications). MetHb were estimated on day 1 and day 10 of treatment of all the cases of study and control group. The findings were critically analyzed, correlated with clinical features and severity of cases. The outcome was death or discharge. Patients were examined at least 8 hourly till full recovery or death. Statistical analysis-SPSS-17 software was used for all statistical analysis. Linear variables were compared using 't' test for independent variables. The probability $p < 0.05$ is considered to be significant.

Results

This study included total 60 subjects, out of that 30 patients with malaria taken as cases of study group and 30 were controls. The mean age group of study was 41.66 years. Out of 30 confirmed cases of malaria 21 (70 %) were complicated and 9 (30 %) were uncomplicated. The presenting features of complicated cases were fever 21 (100 %), anemia 17 (80.95 %) jaundice 11 (52.38 %), renal failure 12 (57.14 %), hypoglycemia 4 (19.04 %), coma/convulsion 5 (23.8 %), shock 3 (14.28 %), acidosis 5 (23.8 %), acute respiratory distress syndrome 2 (9.58 %), bleeding/disseminated intravascular coagulation 1 (4.76 %) and haemoglobinuria 1 (4.76 %). Anemia with jaundice ($n = 5$), anemia with renal failure ($n = 5$) were most common combinations. Three or more organ dysfunction was seen in five patients in whom most have anemia, renal failure and jaundice. The mean MetHb levels were found to be highest in patients having all the complications like anemia, jaundice, renal failure, hypoglycemia group (3.52% on day 1 and 22.85% on day 10) and least found in anemia with jaundice group (1.45% on day 1 and 1.62% on day 10). Mean MetHb on day 1 was $2.038 \pm 1.684 \%$ for study group and $0.795 \pm 0.2831 \%$ for controls, while on day 10 it became $7.69 \pm 2.843 \%$ for study group and $0.685 \pm 0.235 \%$ for controls. When compared, difference of score is significant on day 1 (p value = 0.0002) while on day 10 it became even more significant (p value = 0.0005) as in Table 1. The mean MetHb with complicated malaria on day 1 was $2.55 \pm 1.75 \%$ and with uncomplicated malaria $0.83 \pm 0.49 \%$ and on day 10 it became 10.69 ± 8.19 and $2.34 \pm 1.31 \%$, respectively and compared using unpaired 't' test for significance. Difference of score was found to be significant both on day 1 (p value = 0.008) and day 10 (p value = 0.038) as shown in Table 1. Similarly the mean of MetHb level of cases who survived on day 1 was $1.192 \pm 0.935 \%$ while on day 10 it was $1.9058 \pm 0.822 \%$ and for cases who died, it was

Table 1 Comparisons of methaemoglobin levels in cases of complicated and uncomplicated malaria and controls

Results	n (%)	Mean	SD	SEM
Day 1				
Cases (total)	30	2.038	1.684	0.307
Complicated cases	21	2.55	1.75	0.38
Uncomplicated cases	9	0.836	0.49	0.49
Control	30	0.795	0.2831	0.051
Day 10				
Cases (total)	25	7.69	2.843	1.969
Complicated cases	16	10.69	8.19	2.8
Uncomplicated case	9	2.34	1.31	0.77
Control	27	0.685	0.235	0.453
<i>t</i> test equality of means				
Day	t score	Df	p value	
Day 1	3.989	58	0.0002	
Day 10	3.7	50	0.0005	

Table 2 Comparison of methaemoglobin levels among cases that survived and died

Results	n (%)	Mean	SD	SEM
Within day 1				
Survived	17 (56)	1.192	0.935	0.2268
Died	13 (43)	3.144	1.829	0.5073
Day 2–10				
Survived	9 (30)	1.9058	0.822	0.4419
Died	8 (27)	19.982	8.406	2.972

3.144 ± 1.829 % on day 1 and 19.982 ± 8.406 % on day 10. When compared, the difference of scores for survived cases and died cases is significant on day 1 (p value = 0.0007) and became very significant on day 10 (p value = 0.0001) as shown in Table 2. A positive and direct proportionate correlation was observed between the mean level of MetHb and degree of parasitemia and magnitude of hemolysis (serum LDH, LFT and anemia). Patients with highest grade (+++++) Parasitemia ($n = 20$) had mean day 1 and day 10 MetHb level as 2.55 and 8.65 % while the corresponding value in patients with less parasitemia (Grade +, ++, +++) were 1.5 and 1.9 %, respectively. While the corresponding near value of serum LDH and Hb were 715 u/L, 8.6 % on day-1, 916 UL, 7.5 % on day 10 and 660 U/L, 9.9 % on day-1 670 U/L, 8.5 % on day 10, respectively.

Discussion

The present work “study of methaemoglobin in malaria patients” included 30 confirmed cases of malaria which

form study group and 30 cases of non-febrile illness which form the control group. Out of 30 cases of malaria 22 (73.33 %) cases were males and 8 (26.66 %) were females. Among 30 controls 21 (70 %) were males and 9 (30 %) were females. It could be that males are more frequently exposed to the risk of acquiring malaria than females because of their more outdoor activities. Further female Indians are better clothed than males. Kochar et al. [8] in their series on malaria in Rajasthan observed that 50.8 % were males and 49.2 % were females. The mean age of cases was 41.66 years and mean age of controls were 39.86 years with no significant difference. Male female ratio in patient group and control group were 2.75:1 and 2.33:1, respectively.

The mean MetHb levels on day 1 were 2.038 ± 1.684 % for study group and 0.795 ± 0.2831 % for control group. Following 10 days it was 7.69 ± 2.843 % for study group and 0.685 ± 0.235 % for control group. Difference in scores were significant both at day 1 of treatment (p value = 0.0002) and day 10 of treatment (p value = 0.0005). This study is consistent with finding of study done by Akompong et al. [9] who found level of methaemoglobinemia was between 20 and 42 % in patients whose red cells were infected with malaria parasites while only 0.5–1.0 % in patients whose red cells were not infected with malaria parasites. Therefore, these reports indicate that malaria parasite induced raised levels of methaemoglobin.

While compared complicated malaria patients with uncomplicated malaria the difference of score was found to be significant both on day 1 (mean 2.55 vs 0.83, p value = 0.008) and day 10 (mean 8.19 vs 1.31, p value = 0.038). This finding suggests that there is significant difference in MetHb level in blood of malaria patients having complications than uncomplicated patients on day of admission and also after 10 days of treatment. Similarly for survived and died patients the score was significant on day 1 (mean 1.19 vs 3.14, p value = 0.0007) and became very significant on day 10 (mean 1.9 vs 19.98, p value = 0.0001). In addition the level of MetHb was direct proportionate to the degree of parasitemia and hemolysis. All the factors indicate that as the severity of malaria increases there will be increase in level of MetHb in blood of malaria patient. Raised MetHb levels were found with increase in severity and increase in complications in malaria patients. However Uko et al. [10] found erythrocyte obtained from blood sample of malaria patients exhibited significantly ($p < 0.05$) higher levels of methaemoglobin concentrations than those of non-malaria individuals, irrespective of their genotype and gender. In a similar manner, *P. falciparum* infected erythrocyte of malaria male individuals contained significantly ($p < 0.05$) higher methaemoglobin concentration than the female group. Furthermore, Anstey et al. [11] documented elevated MetHb concentration of Tanzanian

children with severe and uncomplicated malaria. The degree of methaemoglobinaemia was correlated with disease severity and severity of anemia. Mean methaemoglobin levels in children with asymptomatic parasitemia, uncomplicated malaria, severe anemia, cerebral malaria with complete recovery and cerebral malaria with death were 3.3, 4.1, 5.6, 4.7 and 5.8 %, respectively; the mean levels in those with clinical disease were significantly higher than those in healthy controls (2.0 %). Methaemoglobinaemia >10 % was found in 5.4, 16.7, 12.5 and 22.2 % of those with uncomplicated malaria, severe anemia, cerebral malaria with complete recovery and cerebral malaria with death, respectively. These suggest that increased MetHb level in blood may be associated with decreased oxygen carrying capacity of blood i.e. increase in hypoxia and vice versa which is ultimately associated with increased mortality. This result is consistent with the fact that reduction in oxygen carrying capacity of blood in malaria is not only due to a decrease in the quantity of haemoglobin, but also due to a qualitative impairment in the ability of remaining haemoglobin to carry oxygen [11]. It can be presumed that the level of MetHb is more in complicated malaria due to involvement of more number of erythrocyte population than in uncomplicated malaria. Malaria parasite induces raised level of MetHb due to rapid oxidation of Hb. Higher rise of MetHb on day 10, observed in complicated and more severe cases support this explanation. Another aspect of the level of MetHb in malaria setting needs consideration. Studies have proved that MetHb contribute to both morbidity and mortality by hypoxia due to the decrease oxygen binding capacity of methemoglobin, as well as the increase oxygen binding affinity of other subunits in the same hemoglobin molecule which prevents them from releasing oxygen at normal tissue oxygen level. The impact (both morbidity and mortality) of reduction of MetHb level or prevention of production of MetHb by various drugs like methylthioninium chloride (methylene blue) and ascorbic acid along with other standard of care in randomized trials in malaria patients [12]: an exciting research should be undertaken keeping the magnitude of importance in our country.

Conclusion

It is concluded that complicated malaria is associated with significant increase in MetHb in comparison to uncomplicated malaria and control group. Its level is well corroborated with severity of clinical presentation, degree of

parasitemia and mortality. Thus this may be considered as a prognosticator at time of presentation as well as during treatment. Further studies are needed to confirm this and whether reduction of MetHb along with other standard therapy is beneficial or not?

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