J Vector Borne Dis 46, June 2009, pp. 100–108

Malarial anaemia and nitric oxide induced megaloblastic anaemia: a review on the causes of malarial anaemia

Prasanna Pradhan

Regional Medical Research Centre (ICMR), Bhubaneswar, India

Abstract

Direct destruction and ineffective erythropoesis does not adequately explain the cause of anaemia in malaria. It is possible that there are more other mechanisms involved besides the causes described till date in malarial anaemia. The effect of NO on erythropoesis and a major haematological abnormality (microcytic/normocytic/megaloblastic picture) can significantly be observed on repeated exposure. In addition, NO can inhibit the enzyme *methionine synthase* so functional vit B_{12} deficiency state may occur which can lead to megaloblastic anaemia. This review will focus on causation of malarial anaemia and nitric oxide induced megaloblastic anaemia.

Key words Dyserythropoesis – immunological destruction of RBC – malarial anaemia – megaloblastic anaemia – methionine synthase inhibition – NO induced anaemia – vit B_{12} deficiency

Introduction

Malaria is caused by obligate intracellular parasites, which live in host erythrocytes and remodel these cells to provide optimally for their own needs. It is a major public health problem in tropical areas, and it is estimated that malaria is responsible for 1 to 3 million deaths and 300–500 million infections annually. The vast majority of morbidity and mortality from malaria is caused by infection with *Plasmodium falciparum,* although *P. vivax, P. ovale,* and *P. malariae* are also responsible for human infections. A lack of acquired immunity to *P. falciparum* malaria in young children appears to underlie the high rates of morbidity and mortality from malaria in areas of sub-Saharan Africa where malaria is endemic[1]. Although the molecular mechanisms responsible for effective malarial immunity remain elusive, production of nitric oxide (NO) appears to be an important marker and potential mediator of disease severity[2]. Also, NO mediates a diverse array of physiologic and pathologic processes, and appears to be an important mediator of the protective immune response to all stages of *Plasmodium* infections[3]. This review will focus on malarial anaemia and the relationship of nitric oxide with malarial anaemia.

Anaemia in malaria

Malarial anaemia is thought to arise from both decreased red blood cell (RBC) production and increased RBC destruction. Destruction of RBCs can occur as a result of parasite invasion and replications. The pathophysiology of severe anaemia is a complex but relatively neglected area of study. Certainly, malaria gives ample reasons for both increased destruction and reduced production of red cells.

Direct and immunological destruction: Red blood cells are destroyed as parasites complete their growth cycle, although some parasites may be removed from erythrocytes as immature ring forms by phagocytic cells[4]. Infected erythrocytes may also be phagocytosed by macrophages following opsonization by immunoglobulins and/or complement components. Other effector cells and mechanisms are less well-defined but may include antibody-dependent cytotoxicity and natural killer (NK) cells. The survival of

uninfected erythrocytes is also reduced. Mathematical modeling of haematologic data from experimental human *P. falciparum* infections[5] as well as analysis of clinical data from endemic areas[6], have suggested that up to 12 uninfected RBCs (uRBCs) are lost for every infected RBC.

Thus, it is widely accepted that direct destruction of RBCs following parasitization cannot account for the degree of anaemia observed during malaria infection, suggesting that the destruction of uRBCs is the major cause of haemoglobin (Hb) loss[7]. Malarial anaemia may be mediated in part by immunopathologic processes[8]. The activity and the number of macrophages are increased in malarial infection. Moreover, the signals for recognition of uninfected erythrocytes for removal by macrophages are enhanced. Uninfected erythrocytes bind increased amount of immunoglobulin and/or complement as detected in the direct antiglobulin test (DAT or Coomb's Test)[9,10]. The specificity of the immunoglobulins on the surface of the red cells has remained controversial. These antibodies do not have a particular specificity but are more likely to represent immune complexes absorbed onto the surface of red blood cells by complement receptors including CR1 (CD35)[11]. Reticulocytopenia has been observed in numerous clinical studies of malarial anaemia.

Sometimes in asymptomatic malaria, anaemia is frequently out of proportion to the low level of parasitaemia found, suggesting that it is not mediated simply by direct destruction/haemolysis of parasitized red blood cells. The anaemia of asymptomatic parasitaemia is important since children may later develop severe anaemia, both with and without subsequent episodes of acute clinical malaria[12].

Due to suppression of erythropoesis: The histopathological study of the bone marrow of children with malarial anaemia shows impaired bone marrow response[13–15], erythroid hyperplasia, with dyserythropoiesis, cytoplasmic and nuclear bridging, and irregular nuclear outline[16]. The functional abnormality has not been defined, but an increased proportion of the erythroid progenitors are found in the G2 phase compared with normal controls. The prime candidates for the host factors mediating dyserythropoiesis have been growth factors and cytokines. Serum erythropoietin (Epo) was appropriately raised in a study and EPO production is robust and correlates inversely with the degree of anaemia[15,17]. Acute haemolysis and impaired bone marrow response are important in this setting. Parasitaemia and NO production measured in cross-sectional studies on any one day will not necessarily reflect mean parasitaemia and mean levels of NO production to which the bone marrow has been exposed in preceding weeks. Although there was no history of fever in the two weeks prior to recruitment, it is possible that the haemoglobin levels measured could have also been influenced by past intercurrent episodes of acute clinical malaria[18].

The concentrations of tumor necrosis factor-α (TNF-α) and interferon (IFN)-γ have been correlated with the severity of the disease[19] and high levels of TNF-α have been shown to suppress erythropoiesis.These cytokines may also contribute to reduced production of Epo and to increased erythrophagocytosis. The possibility has been raised that high levels of the Th2-type cytokine interleukin-10 (IL-10) might prevent the development of severe malarial anaemia. Low levels of IL-10 have been described in African children with severe malarial anaemia[20]. However, the mechanism of protection from anaemia by IL-10 is unclear. The hypothesis that parasite products directly stimulate the production of inflammatory cytokines, including TNF-α, has been widely promoted.

Nitric oxide is an inhibitor of erythropoisis[21]. Cytokine-induced NO is known to decrease human erythropoiesis, and NO is likely an important mediator of the anaemia of chronic disease in humans[22]. Although increased NO production appears to be associated with protection against malaria in the Gabonese children that were previously investigated, elevated levels of NO can suppress erythropoiesis[23] and induce apoptosis in cultured $CD34^+$ cells[24]. *In vitro* studies show that tumor necrosis factor TNF-

α and interferon-γ induced suppression of human haematopoiesis is in part mediated by NO[25].

The glycosylphosphatidylinositol (GPI) anchor of malarial membrane proteins may cause cellular dysfunction, but a role for this toxin in dyserythropoiesis remains to be established[26]. Other toxic products may exist. During its blood stage, the malaria parasite proteolyses host haemoglobin, releasing heme as a by-product. β-Haematin forms as a crystalline cyclic dimer of oxidized heme and is complexed with protein and lipid products as malarial pigment or haemozoin. The function of monocytes and macrophages is severely inhibited after ingestion of haemozoin. Here, the biologically active moieties may be lipoperoxides such as 4-hydroxynonenal (4-HNE) and 15 (R, S) hydroxyeicosatetraenoic acid (HETE) produced by oxidation of membrane lipids[27]. Their effect on other cellular functions, such as erythropoiesis, has not been established. Anaemia in falciparum malaria is clearly multifactorial and there is a strong argument that erythrocyte destruction and ineffective erythropoiesis play equal parts in the etiology of malarial anaemia.

In the above conditions, anaemia is typically normocytic and normochromic, with a notable absence of reticulocytes, although microcytosis and hypochromia may be present due to the very high frequency of alpha and beta thalassemia traits and/or iron deficiency in many endemic areas[28].

Due to defect in one carbon transfer by NO: Nitric oxide (NO) is produced by most cell types and regulates a diverse array of biological functions[29–31]. NO is known to react with heme proteins, porphyrins, and cobalamins to form nitrosyl-metal complexes[32–36]. NO has been reported to inhibit methionine synthase activity *in vitro*[37–39], and it might be expected to bind to the cobalt in cobalamin because, first, NO binds tightly to the iron in heme[40]; second, ferrous heme and cbl(III) are isoelectronic; and third, in both heme and cobalamin, the metal ion is coordinated to four in-plane nitrogen atoms of a tetrapyrrole ring and has two out-of-plane ligands[41]. NO has a remarkably high affinity for ferrous heme with a binding constant on the order of 10^{12} to 10^{14} M^{-1}, and NO also binds to ferric heme[42]. Iron and cobalt are transition metals adjacent in the periodic table, and the porphyrin ring of heme and the corrin ring of cobalamin are both substituted tetrapyrrole rings[43]. Thus, it is not surprising that NO binds to the cobalt in cobalamin, and it is observed that NO reacts with all three valency states of cobalamin[44].

Cobalamin exists in two metabolically active forms, identified by alkali group attached to sixth coordinated position of cobalt atom: methylcobalamin and adenosylcobalamin. The therapeutic preparation is Vitamin B_{12} (cyanocobalamin) which has no known physiologic role and must be converted to biologically active form. Methylcobalamin is the form required for methionine synthase in folate metabolism[45]. In methylcobalamin, a methyl group is bonded to cobalt in the upper axial position, and the lower axial position is occupied by a nitrogen of the dimethylbenzimidazole nucleotide substituent of the corrin ring. During turnover, the cobalamin cofactor of methionine synthase shuttles between methylcobalamin and cob(I) alamin (which contains a pair of electrons in the dz2 orbital oriented perpendicularly to the plane of the corrin ring). The enzyme-bound cob(I) alamin can be oxidized to cob(II) alamin, with a single electron in the dz2 orbital, or to cob(III) alamin[46]. Adenosylcobalamin is required for conversion of methylmalonyl CoA to succinyl CoA and abnormality in this will lead to fatty acid accumulation[45]. Methionine synthase is poised at the point of convergence of two major biosynthetic pathways: the tetrahydrofolate-dependent pathway for biosynthesis of methyl groups and the homocysteine biosynthetic pathway in prokaryotes. However, in mammals on the other hand, are unable to synthesize homocysteine *de novo*; rather they use methionine synthase to regenerate methionine from homocysteine to provide one-carbon units for S-adenosyl-methionine (Ado-Met)-dependent methylation reactions (Fig. 1)[46].

Some researchers have proved that NO reacts rapidly and irreversibly with cbl(I)[44] which could oxidize

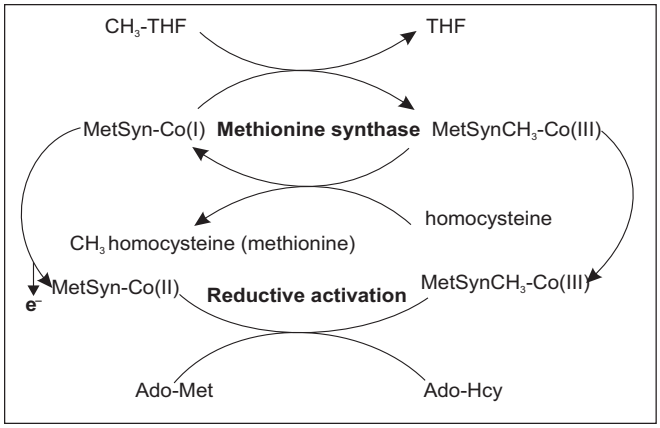

Fig. 1: Transfer of the methyl group of methyltetrahydrofolate (CH_3-THF) to homocysteine via methionine synthase-methylcobalamin [MetSyn-CH_3-Co(III)] as an intermediate methyl carrier. The reductive methylation in the lower part of the scheme is the mechanism by which *S*-adenosylmethionine (Ado-Met) together with an electron reactivates the enzyme after oxidative inactivation. Ado-Hcy, *S*-adenosylhomocysteine

cbl(I) to cbl(II) and therefore, interfere with the cbl(I)→ cbl(III) cycle that is an essential part of the methionine synthase reaction[47]. Marius *et al*[48] have observed that NO is able to diminish the cofactor activity of cobalamin for methionine synthase. Inhibition is mediated by NO-cobalt interactions. Because nitrosylcobalamin can transfer NO to glutathione. It is also possible that the decrement in cofactor ability of cobalamin is caused by modification of the substrate homocysteine to S-nitroso-homocysteine formation (by NO transferred from nitrosylcobalamin to homocysteine)[48]. Nicolaou *et al*[49] have reported NO-mediated inhibition of methionine synthase (Fig. 2). In some experiments it has been seen that NO inhibits methionine synthase activity *in vivo* and that NO produced by three different pharmacological agents or produced physiologically by rat C6 glioma cells inhibits carbon flow through the folate pathway[44]. Surprisingly, it binds to all three valency states of cobalamin and mechanism of methionine synthase inhibition appears to be similar to that of N_2O[44]. However, conflicting results have been published concerning NO binding to cobalamin like, NO binds only to divalent cobalamin (*i.e.* cbl[II])[50], and some found that NO binds to both cbl(II) and (III)[48].

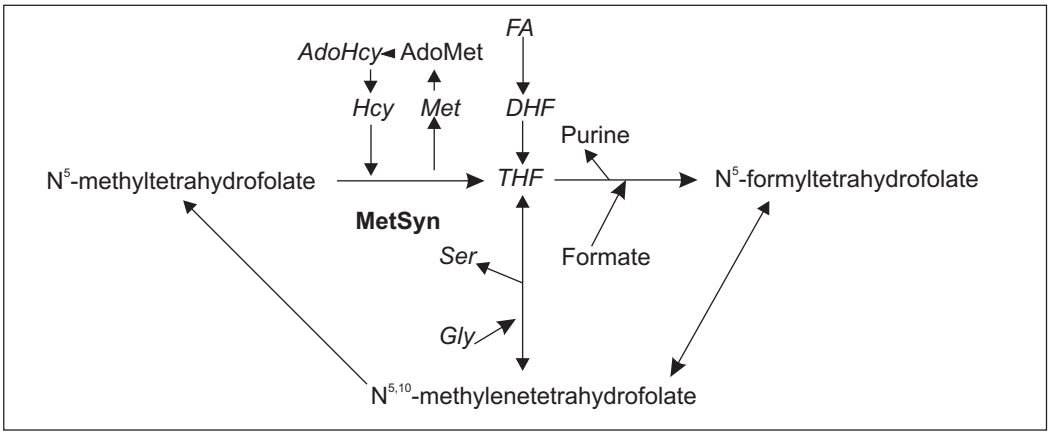

Fig. 2: **Folate-methionine reactions**. Folic acid (*FA*) is reduced to tetrahydrofolate (*THF*) via dihydrofolate (*DHF*). Formate enters the folate pathway by combining with tetrahydrofolate to form 10-formyltetrahydrofolate, which can be reversibly converted to 5, 10-methyltetrahydrofolate and to 5,10-methylenetetrahydrofolate. The latter compound is converted irreversibly to 5-methyltetrahydrofolate, the major intracellular storage form of folates. Methionine synthase transfers the methyl group of 5-methyltetrahydrofolate to homocysteine (*Hcy*) via a cobalamin intermediate and thereby regenerates free tetrahydrofolate. Methionine (*Met*) can be converted to AdoMet, the main intracellular source of methyl groups for transmethylase reactions. A transsulfuration cycle is completed when *S*-adenosylhomocysteine (*AdoHcy*) is converted to homocysteine. Carbons in positions 2 and 8 of the purine ring are derived from 10-formyltetrahydrofolate during *de novo* purine synthesis, and serine (*Ser*) can be synthesized from glycine (*Gly*) using a carbon from 5,10-methylene tetrahydrofolate

And some, initially reported that NO binds to cbl(III) and oxidizes cbl(II) to cbl(III) but subsequently concluded that NO does not react with either species[51].

In an experiment by Idrees *et al*[44] NO donors decreased [14C] methyl tetrahydrofolate incorporation into protein; and homocysteine reduced the effectiveness of PAPA-NONOate (propylamine prompylamine NONOate) as an inhibitor of *de novo* purine nucleotide synthesis. NO, is known to inhibit ribonucleotide reductase[52], which could decrease DNA synthesis and thereby cause purine nucleotides to accumulate; the latter could inhibit *de novo* purine synthesis by a feedback mechanism.

Relative high levels of NO may be produced *in vivo* in humans in various conditions, including infection, septic shock, and trauma, It is clear that humans can be induced to produce increased amounts of NO *in vivo* with infection and shock[53] and when receiving IL-2 treatment for cancer[54,55]. The cells producing the excess NO *in vivo* in these conditions are not known. Mononuclear phagocytes, hepatocytes, smooth muscle cells, endothelial cells, and/or other cells could be overproducing NO. In these circumstances, it is possible that the NO might diminish the enzyme cofactor abilities of cobalamins and produce a functional vitamin B_{12} deficiency state.

Nitric oxide also alters cellular iron metabolism, and it likely contributes (through its effects on iron metabolism) to the anaemia of chronic diseases[22]. Iron deficiency itself is a major cause of anaemia in malaria-endemic areas[56].

Discussion

Some workers defined two forms of malaria-associated anaemia predominate: anaemia associated with (a) acute clinical episodes of malaria (or a history of such episodes), and (b) anaemia associated with the chronic, intermittent, asymptomatic, low-grade parasitaemiae found in 100% of children in endemic areas[57–60]. In this latter group with asymptomatic parasitaemia, the anaemia is frequently out of proportion to the low level of parasitaemia found, suggesting that it is not mediated simply by direct destruction/haemolysis of parasitized red blood cells. The anaemia of asymptomatic parasitaemia is important since children may later develop severe anaemia, both with and without subsequent episodes of acute clinical malaria. So direct destruction and ineffective erythropoiesis does not adequately explain the cause of anaemia in malaria. It is possible that there are more other mechanisms involved besides the above described for causation of malarial anaemia.

NO is rapidly oxidized to the stable inorganic nitrogen oxides, nitrite and nitrate *in vivo*[47,61]. Nitrite, rather than nitrite plus nitrate, is believed to be the product of NO in oxygenated water[62]. Haemoglobin possesses anion binding sites that may retain nitrite, raising concerns that the measured NO levels may be overestimated as a result of conversion of haemoglobin-bound nitrite to NO[63]. The majority of studies examining NO production in malaria-exposed adults have reported NO metabolite levels in the setting of clinical disease[64–67]. Most of these studies did not control for dietary nitrate ingestion[64,65] or altered nitrate handling in renal impairment[64,67]. Very few studies have reported NO metabolite levels in asymptomatic malaria-exposed adults[64,68], and none of these controlled for the confounding effect of dietary nitrite-plus-nitrate (NOx) ingestion. Also NO is produced in response to severity and some other cause (inflammation, super added infection, shock etc)[53] as the disease progress. So it is not specific that only malaria induced NO will inhibit methionine synthase and direct cause of ineffective erythropoiesis in malaria endemic area. Also we can infer that in chronic inflammatory conditions or infections, megaloblastic anaemia can be seen.

Any adverse influence of NO on haematopoiesis is likely to result from the effects of sustained NO production over days-weeks in response to chronic parasitaemia. Production of NO is likely to fluctuate longitudinally in response to the longitudinal fluctuations in parasite density known to occur in asymptomatic parasitaemia[69]. Parasitaemia and NO

production measured in cross-sectional studies on any one day will not necessarily reflect mean parasitaemia and mean levels of NO production to which the bone marrow has been exposed in preceding weeks. Although there was no history of fever in the two weeks prior to recruitment, it is possible that the haemoglobin levels measured could also have been influenced by past intercurrent episodes of acute clinical malaria[18]. Thus the effect of NO on erythropoiesis and a major haematological abnormality (microcytic[28]/normocytic/megaloblastic picture) can significantly be observed on repeated exposure and in asymptomatic cases after repeated exposure in endemic areas. And sudden burst of NO in 4–5 days may not predispose to megaloblastic or microcytic picture.

The majority of studies examining NO production in malaria-exposed adults have reported NO metabolite levels in the setting of clinical disease[64–68], reflecting altered NO production in clinical malaria. NO could bind to the methyl ligand binding site of cbl(III), thereby preventing or diminishing formation of the CH3-cbl(III) intermediate in the methionine synthase reaction. Second, NO could oxidize cbl(I) to cbl(II) and therefore, interfere with the cbl(I)→cbl(III) cycle that is an essential part of the methionine synthase reaction[47]. Although NO, like N_2O, appears to inhibit methionine synthase activity by interacting with cbl(I), the nature of enzyme inhibition may be quite different between the two gases. While N_2O damages the protein by producing OH-radical, with depletion of cobalamin being a secondary cause, NO should prevent only regeneration of the CH3-cbl(III) prosthetic group. If rapid burst of NO by iNOS occurs then it is possible that like N_2O acute megaloblastosis with normocytic normochromic anaemia like features can be observed. But some researchers found no evidence of NO binding to either cbl(II) or (III)[70] and some initially reported that NO binds to cbl(III) and oxidizes cbl(II) to cbl(III) but subsequently concluded that NO does not react with either species[71]. Again it has been seen that aquocobalamin (H,O-Cbl (but not cyanocobalamin [CN-Cbl], methylcobalamin [Me-Cbl], or adenosylcobalamin [Ado-Cbl]) reacts with NO[48]. So the causation of megaloblastic anaemia in malaria remains elusive and can be confirmed by measuring methionine synthase level in blood and peripheral blood smear in chronic asymptomatic cases where sustained NO production is evident.

References

1. Breman JG, Egan A, Keusch GT. The intolerable burden of malaria: a new look at the numbers. *Am J Trop Med Hyg* 2001; *64:* iv–vii.
2. Kremsner PG, Winkler S , Wildling E, Prada J, Bienzle U, Graninger W, Nussler AK. High plasma levels of nitrogen oxides are associated with severe disease and correlate with rapid parasitological and clinical cure in *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 1996; *90:* 44–7.
3. Anstey NM, Weinberg JB, Granger DL. Nitric oxide and malaria. In: Fang F, editor. *Nitric oxide and infection.* New York: Plenum Publishing Corp 1999; p. 311–41.
4. Angus BJ, Chotivanich K, Udomsangpetch R, White NJ . *In vivo* removal of malaria parasites from red blood cells without their destruction in acute falciparum malaria. *Blood* 1997; *90:* 2037.
5. Jakeman GN, Saul A, Hogarth WL, Collins WE. Anaemia of acute malaria infections in non-immune patients primarily results from destruction of uninfected erythrocytes. *Parasitology* 1999; *119* (pt 2): 127–33.
6. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, ter Kuile F, *et al*. Factors contributing to anaemia after uncomplicated falciparum malaria. *Am J Trop Med Hyg* 2001; *65:* 614–22.
7. Ekvall H. Malaria and anaemia. *Curr Opin Hematol* 2003; *10:* 108–14.
8. Schofield L, Grau GE. Immunological processes in malarial pathogenesis. *Nat Rev Immunol* 2005; *5:* 722–35.
9. Facer CA, Bray RS, Brown J. Direct Coombs antiglobulin reactions in Gambian children with *Plasmodium falciparum* malaria. I: incidence and class specificity. *Clin Exp Immunol* 1979; *35:* 119.
10. Abdalla SH, Kasili FG, Weatherall DJ. The Coombs direct antiglobulin test in Kenyans. *Trans R Soc Trop Med Hyg* 1083; *77:* 99.
11. Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. Red cell surface changes and erythrophagocytosis in children with severe *Plasmodium falciparum* anaemia. *Blood* 2000; *95:* 1481.

12. Greenwood B. Asymptomatic malaria infections—do they matter? *Parasitol Today* 1987; *3:* 206–14.

13. Phillips R, Pasvol G. Anaemia of *Plasmodium falciparum* malaria. *Baillieres Clin Haematol* 1992; *5:* 315–30.

14. Davis T, Krishna S, Looareesuwan S, Supanaranond W, Pukrittayakamee S, Attatamsoonthorn K, White NJ. Erythrocyte sequestration and anaemia in severe falciparum malaria. Analysis of acute changes in venous haematocrit using a simple mathematical model. *J Clin Invest* 1990; *86:* 793–800.

15. Kurtzhals JA, Rodrigues O, Addae M, Commey JO, Nkrumah FK, Hviid L. Reversible suppression of bone marrow response to erythropoietin in *Plasmodium falciparum* malaria. *British J Haematol* 1997; *97:* 169–74.

16. Abdalla SH. Hematopoiesis in human malaria. *Blood Cells* 1990; *16:* 401.

17. Villeval JL, Lew A, Metcalf D. Changes in hemopoietic and regulator levels in mice during fatal or nonfatal malarial infections. I: erythropoietic populations. *Exp Parasitol* 1990; *71:* 364–74.

18. Nicholas M Anstey, Donald L Granger, Mushtaq Y Hassanali, Esther D Mwaikambo, Patrick E Duffy, Brice J Weinberg. Nitric oxide, malaria and anaemia: inverse relationship between nitric oxide production and haemoglobin concentration in asymptomatic, malaria-exposed children. *Am J Trop Med Hyg* 1999; *61*(2): 249–52.

19. Grau GE, Taylor TE, Molyneux ME, Wirima JJ, Vassalli P. Tumor necrosis factor and disease severity in children with falciparum malaria. *N Engl J Med* 1989; *320:* 1586.

20. Kurtzhals JA, Adabayeri V, Goka BQ, Akanmori BD, Oliver-Commey JO, Nkrumah FK, Behr C, Hviid L. Low plasma concentrations of interleukin 10 in severe malarial anaemia compared with cerebral and uncomplicated malaria. *Lancet* 1998; *351:* 1768 [errata in *Lancet* 1998; *352* (9123): 242; and 1999; *353* (9155): 848].

21. De Sousa K, Silva MS, Tavira LT. Variation of nitric oxide levels in imported *Plasmodium falciparum* malaria episodes. *African J Biotechnol* 2008; *7*(6): 799.

22. Domachowske JB. The role of nitric oxide in the regulation of cellular iron metabolism. *Biochem Mol Med* 1997; *60:* 1–7.

23. Shami PJ, Weinberg JB. Differential effects of nitric oxide on erythroid and myeloid colony growth from $CD34^+$ human bonemarrow cells. *Blood* 1996; *87:* 977–82.

24. Reykdal S, Abboud C, Liesveld J. Effect of nitric oxide production and oxygen tension on progenitor preservation in *ex vivo* culture. *Exp Hematol* 1996; *27:* 441–50.

25. Maciejewski JP, Selleri C, Sato T, Cho HJ, Keefer LK, Nathan CF, Young NS. Nitric oxide suppression of human hematopoiesis *in vitro*—contribution to inhibitory action of interferon-gamma and tumor necrosis factor-alpha. *J Clin Invest* 1995; *96:* 1085–92.

26. Tachado SD, Gerold P, Schwarz R, Novakovic S, McConville M, Schofield L. Signal transduction in macrophages by glycosylphosphatidylinositols of *Plasmodium*, Trypanosoma, and Leishmania: activation of protein tyrosine kinases and protein kinase C by inositolglycan and diacylglycerol moieties. *Proc Natl Acad Sci USA* 1997; *94:* 4022.

27. Urban BC, Roberts DJ. Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. *Curr Opin Immunol* 2002; *14:* 458.

28. Newton CR, Warn PA, Winstanley PA, Peshu N, Snow RW, Pasvol G, Marsh K. Severe anaemia in children living in a malaria endemic area of Kenya. *Trop Med Int Health* 1997; *2:* 165–78.

29. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 1991; *43:* 109.

30. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; *329:* 2002–12 (Review).

31. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. *Ann Rev Pharmacol Toxicol* 1990; *30:* 535–60.

32. Kaczka EA, Wolf DE, Kuehl FA Jr, Folkers K. Vitamin BIZ. XVI: Modification of cyano-cobalamin. *J Am Chem Soc* 1951; *73:* 3569.

33. Rochelle LG, Morana SJ, Kruszyna H, Russell MA, Wilcox DE, Smith RP. Interactions between hydroxocobalamin and nitric oxide (NO)—evidence for a redox reaction between NO and reduced cobalamin and reversible NO binding to oxidized cobalamin. *J Pharmacol Exp Ther* 1995; *275:* 48.

34. Alayash AI, Fratantoni JC, Bonaventura C, Bonaventura J, Cashon RE. Nitric oxide binding to human ferrihemoglobins crosslinked between either alpha or beta subunits. *Arch Biochem Biophys* 1993; *303:* 332.

35. Sharma VS, Traylor TG, Gardiner R. Reaction of nitric oxide with heme proteins and model compounds of hemoglobin. *Biochemistry* 1987; *26:* 3837.

36. Wade RS, Castro CE. Redox reactivity of iron (II1) porphyrins and heme proteins with nitric oxide. Nitrosyl transfer to carbon, oxygen, nitrogen, and sulfur. *Chem Res Toxicol* 1990; *3:* 289.

37. Brouwer M, Chamulitrat W, Ferruzzi G, Sauls DL,

Weinberg JB. Nitric oxide interaction with cobalamins: biochemical and functional consequences. *Blood* 1996; *88:* 1857–64.

38. Nicolaou A, Warefield CJ, Kenyon SH, Gibbons WA. *Eur J Biochem* 1997; *244:* 876–82.

39. Nicolaou A, Kenyon SH, Gibbons JM, Ast T, Gibbons WA. *In vitro* activation of mammalian methionine synthase by nitric oxide. *Eur J Clin Invest* 1996; *26:* 167–70.

40. Kharitonov VG, Bonaventura J, Sharma VS. Interaction of nitric oxide with heme protein using UV-VIS spectroscopy. In : Feelisch M, Stamler JS, editors. *Methods in nitric oxide research*. New York: John Wiley & Sons Inc 1996; p. 40–5.

41. Ludwig ML, Matthews RG. Structure based perspective on B12 dependant enzyme. *Annu Rev Biochem* 1997; *66:* 269–313.

42. Traylor TG, Sharma VS. Why NO? *Biochemistry* 1992; *31:* 2847–9.

43. *Wintrobe's clinical hematology*, X edn. Baltimore: Williams & Wilkins 1999.

44. Idrees O Danishpajooh, Tanima Gudi, Yongchang Chen, Vladimir G Kharitonov, Sharma Vijay S, Gerry Boss R. Nitric oxide inhibits methionine synthase activity *in vivo* and disrupts carbon flow through the folate pathway. *J Biol Chem* 2001; *276*(29): 27296–303.

45. *Harrison's principles of internal medicine*. Megaloblastic anemias: introduction. XVI edn. Chap 92. New Delhi: Mc-Graw Hill, 2005; p 602.

46. Banerjee Ruma V, Matthews Rowena G. Cobalamin dependant methionine synthase 1990; *4:* 1450–1.

47. Kosaka HK, Lmaizumi K, Imai K, Tyuma I. Stoichiometry of the reaction of oxyhemoglobin with nitrite. *Biochem Biophys Acta* 1979; *581:* 184–8.

48. Marius Brouwer, Chamulitrat Walee, Ferruzzi Giulia, Sauls Derrick L, Weinberg J Brice. Nitric oxide interaction with cobalamins: biomedical and functional consequences. *Blood* 1996; *88:* 1857–64. Available from: *http:// bloodjournal.hematologylibrary.org/*

49. Nicolaou A, Kenyon SH, Gibbons JM, Ast T, Gibbons WA. *In vitro* inactivation of mammalian methionine synthase by nitric oxide. *Eur J Clin Invest* 1996; *26:* 167.

50. Bauer JA. Synthesis, charecterization and nitric oxide release profile of nitrosyl cobalamin : a potential chemotherapeutic agent. *Anti Cancer Drugs* 1998; *9:* 239–44.

51. Kruszyna H, Magyar JS, Rochelle LG, Russell MA, Smith RP, Wilcox DE. Spectroscopic studies of nitric oxide (NO) interactions with cobalamins: reaction of NO with superoxocobalamin(III) likely accounts for cobalamin reversal of the biological effects of NO. *J Pharmacol Exp Ther* 1998; *285:* 665–71.

52. Lepoivre M, Flaman J, Henry Y. Early loss of tyrosyl radical in ribonucleotide reductase of adeno carcinoma cells producing nitric oxide. *J Biol Chem* 1992; *267:* 22994–23000.

53. Ochoa JB, Udekwu AO, Billiar TR, Curran RD, Cerra FB, Simmons RL, Peitzman AB. Nitrogen oxide levels in patients aftertrauma and during sepsis. *Ann Surg* 1991; *214:* 621.

54. Hibbs JB Jr, Westenfelder C, Taintor R, Vavrin Z, Kablitz C, Baranowski RL, Ward JH, Menlove RL, McMuny MP, Kushner JP, Samlowski WE. Evidence for cytokine-inducible nitric oxide synthesis from L-arginine in patients receiving interleukin-2 therapy. *J Clin Invest* 1992; *89:* 867 (published erratum appears in *J Clin Invest* 1992; *90:* 295).

55. Ochoa JB, Curti B, Peitzman AB, Simmons RL, Billiar TR, Hoffman R, Rault R, Longo DL, Urba WJ, Ochoa AC. Increased circulating nitrogen oxides after human tumor immunotherapy: correlation with toxic hemodynamic changes. *J Natl Cancer Inst* 1992; *84:* 864 (published erratum in *J Natl Cancer Inst* 1992; *84:* 1291).

56. Menendez C, Kahigwa E, Hirt R, Vounatsou P, Aponte JJ, Font F, Acosta CJ, Schellenberg DM, Galindo CM, Kimario J, Urassa H, Brabin B, Smith TA, Kitua AY, Tanner M, Alonso PL. Randomised placebo-controlled trial of iron supplementation and malaria chemoprophylaxis for prevention of severe anaemia and malaria in Tanzanian infants. *Lancet* 1997; *350:* 844–50.

57. Newton C, Warn P, Winstanley P, Peshu N, Snow R, Pasvol G, Marsh K. Severe anaemia in children living in a malaria endemic area of Kenya. *Trop Med Int Health* 1997; *2:* 165–78.

58. Abdulla S, Weatherall D, Wickramasinghe S, Hughes M. The anaemia of *P. falciparum malaria. British J Haematol* 1980; *46:* 171–83.

59. Farnet A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. *Am J Trop Med Hyg* 1997; *56:* 538–47.

60. Kitua A, Smith T, Alonso P, Urassa H, Masanja H, Kimario J, Tanner M. The role of low level *Plasmodium falciparum* parasitaemia in anaemia among infants living in an area of intense and perennial transmission. *Trop Med Int Health* 1997; *2*(4): 325–33.

61. Westfelt UN, Benthin G, Lundin S, Stenqvist O, Wennmalm A. Conversion of inhaled nitric oxide to nitrate in man. *British J Pharmacol* 1995; *114:* 1621–4.

62. Butler AR, Flitney FW, Williams DLH. NO, nitrosonium ions, nitoxide ions, nitosothiols and iron-nitrosyls in biology: a chemist's perspective. *Trends Pharmacol Sci* 1995; *16:* 18–22.

63. Imai K. In: Imai K, editor. *Allosteric effects in haemoglobin*. Cambridge, UK: Cambridge University Press 1982; p. 39–45.

64. Kumar Arun C, Das UN. Lipid peroxides, nitric oxide and essential fatty acids in patients with *Plasmodium falciparum* malaria. *Prostaglandins Leukot Essent Fatty Acids* 1999; *61:* 255–8.

65. Dondorp AM, Planche T, de Bel EE, Angus BJ, Chotivanich KT, Silamut K , Romijn JA, Ruangveerayuth R , Hoek FJ, Kager PA, Vreeken J , White NJ. Nitric oxides in plasma, urine, and cerebrospinal fluid in patients with severe falciparum malaria. *Am J Trop Med Hyg* 1998; *59:* 497–502.

66. Taylor AM, Day NP, Sinh DX, Loc PP, Mai TT, Chau TT, Phu NH, Hien TT, White NJ. Reactive nitrogen intermediates and outcome in severe adult malaria. *Trans R Soc Trop Med Hyg* 1998; *92:* 170–5.

67. Torre D, Ferrario G, Matteelli A, Speranza F, Giola M, Pugliese A, Cantamessa C, Carosi G, Fiori GP. Levels of circulating nitrate/nitrite and gamma interferon not increased in uncomplicated malaria. *Infection* 1998; *26:* 301–3.

68. Prada J, Kremsner PG. Enhanced production of reactive nitrogen intermediates in human and murine malaria. *Parasitol Today* 1995; *11:* 409–10.

69. Farnet A, Snounou G, Rooth I, Bjorkman A. Daily dynamics of *Plasmodium falciparum* subpopulations in asymptomatic children in a holoendemic area. *Am J Trop Med Hyg* 1997; *56:* 538–47.

70. Firth RA, Hill HAO, Pratt JM, Thorp RG, Williams RJP. To the chemistry of vitamin B_{12}. Part XI: some further constants. *J Chem Soc* 1969; *A:* 381–6.

71. Rochelle LG, Morana SJ, Kruszyna H, Russell MA, Wilcox DE, Smith RP. Interactions between hydroxocobalamin and nitric oxide (NO): evidence for a redox reaction between NO and reduced cobalamin and reversible NO binding to oxidized cobalamin. *J Pharmacol Exp Ther* 1995; *275:* 48–52.

Corresponding author: Dr Prasanna Pradhan, Regional Medical Research Centre (ICMR), Chandrasekharpur, Nandankanan Road, Bhubaneswar–751 016, India.
E-mail: p.bluedoc@gmail.com

Received: 22 October 2008 *Accepted in revised form:* 4 February 2009