

# Bone Marrow Changes in Human Malaria: A Retrospective Study

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## Abstract

The bone marrow reports of 1966 patients admitted to a provincial teaching hospital between January, 1992 to April, 1995 were retrospectively analyzed . Twenty-six (1.3%) bone marrows showed the presence of malarial parasites. Sixteen (62%) patients had Plasmodium falciparum, 9 (34%) Vivax malaria and one (4%) mixed infection. All these patients gave a history of prolonged illness and had low parasite counts, Plasmodium vivax malaria was not associated with any significant pathology in the bone marrow, except iron deficiency anaemia, The bone marrows with Plasmodium falciparum malaria showed myeloid hyperplasia, erythroid hyperplasia, megaloblastosis and hypoplasia in different proportions. No evidence of dyserythropoiesis was found in this series. The possible mechanisms producing these changes and the factors responsible for the discrepancy in bone marrow findings in different geographical areas are discussed (JPMA 47:137, 1997).

## Introduction

The haematological changes seen in patients with malaria, include anaemia, neutropenia, reactive lymphocytosis, monocytosis, eosinopenia, neutrophil leukocytosis and thrombocytopenia. Of these abnormalities, the most important is anaemia as this is often associated with considerable morbidity particularly in areas with sustained year round infection<sup>1</sup>. The morphological abnormalities affecting the bone marrow have also been reported in Gambian children with severe anaemia and falciparum malaria. Large numbers of erythrocytes are destroyed by parasite replication, but other important elements include depression of erythrocyte production by the bone marrow and phagocytosis of intact erythrocytes both of which are exacerbated by tumor necrosis factor (TNF). The above complication is more likely to be produced by chronic TNF production than by acute bursts of TNF release due to repeated or untreated infection. The purpose of this retrospective study was comparative assessment of bone marrow changes in malaria seen in Pakistan where the disease is endemic with those seen in other areas.

## Patients and Methods

A retrospective analysis of all bone marrow reports in the Lady Reading Hospital Peshawar, was performed by reviewing patient's records. One thousand nine hundred and sixty-six cases were recorded during the period from January, 1992 to April, 1995. The red cell indices, including haemoglobin, red blood cell count, total white blood cell count and platelet count were measured by conventional manual methods. Thick and thin blood films were made and stained with Giemsa stain. The Plasmodium falciparum, asexual forms, were examined and counted against 200 WBC on a thick blood film and the absolute parasite count was calculated from the Observed WBC count as defined by WHO<sup>2</sup>. The indication for bone marrow examination was the diagnosis of various haematological disorders, like iron deficiency anaemia, thrombocytopenia, leukaemia and pyrexia of unknown origin.

The bone marrows were aspirated from posterior superior iliac crests. The smears were stained with May Grunwald and Giemsa stain and in 11(44%) patients staining for iron was done. The degree of megaloblastic change in the erythroid series was recorded and scored as +, ++, and +++. The bone marrow cellularity was reported as hypercellular, hypocellular and normocellular. The erythropoiesis was expressed as normoblastic, hyperplastic and megaloblastic. The bone marrow slides were reviewed by two competent haematologists. The statistical analysis of age, parasite count, haemoglobin and white blood cell count were determined by the Statgraphic program (Version 5.0).

## Results

Of 1966 bone marrow reports, 26 (1.3%) showed the presence of malarial parasites. The total duration of illness ranged from 7 days to 6 months. Sixteen (62%) patients had *Plasmodium falciparum* malaria, 9(34%) were suffering from *Vivax* malaria and one (4%) patient had mixed infection. In nine cases of *Plasmodium vivax* malaria, 4(44%) patients had absent iron stores with no sideroblasts. One (11%) patient had few megaloblasts, while 3 (33%) patients had normal bone marrow. In one patient with *Vivax* malaria marrow showed 20% plasma cells. In sixteen cases with *Plasmodium falciparum* malaria 3 (19%) bone marrows were nonnal, 3 (19%) showed myeloid hyperplasia, 6 (38%) megaloblastic erythropoiesis, 2 (12.5%) had bone marrow hypoplasia, and the remaining 2 (12.5%) showed erythroid hyperplasia. One (6%) case with mixed infection had 30-40% megaloblasts in the bone marrow.

Erythropoiesis in patients with *falciparum* malaria

All these patients with *falciparum* malaria had a low parasite Count and decreased haemoglobin. The patients were divided into two groups i.e., mild anaemia (Hb >8 G%) group 1 and severe anaemia (Hb <8G%) group 2. There were 10 patients in group 1 and 6 in group 2. Two patients in group 1 and 4 patients in group 2 had megaloblastic changes in the bone marrow. The bone marrow cellularity was expressed as normocellular, hypocellular and hypercellular. Two (20%) patients in group 1 and 3 (50%) patients in group 2 had hypercellular marrow, while 2 (12%) patients, one 1mm each group had hypocellular marrow (Table I).

Table I. Bone marrow cellularity and semiquantitative assessment of megaloblasts in the bone marrow of patients with malaria.

S.No.	Species	Hb	No.	Megaloblastic change			Total	B.M. Cellularity		
				+	++	+++		Hypo	Normo	Hyper
1	PF	Hb>8	10	1	1	0	2	1	8	2
	PF	Hb<8	6	-	2	2	4	1	1	3
2	PV	Hb>8	8	-	-	-	0	-	7	-
	PV	Hb<8	1	1	-	-	1	-	2	-
3	Mixed	Hb<8	1	-	1	-	1	-	-	1

Note: The numbers indicate the cases where these features were seen.

Key: +1 indicates few, +2 indicate 30%-40%

+3 indicates more than 40% megaloblasts

PF= *Plasmodium falciparum*

PV= *Plasmodium vivax*

Mixed= PF+PV

Hb=g/dl

The mean and SD values of age, duration of disease, haemoglobin level, platelet count and parasite count are shown in Table II.

**Table II. Age and haematological parameters in Falciparum malaria.**

Parameters	No. of patients	Mean±SD
Age	16	16.5±15.4
WBCx10 <sup>9</sup> /dl	16	8.2±3.93
Hb gm/dl	16	8.8±2.3
Duration of disease in days	16	194.16±131.2
Plateletx10 <sup>9</sup> /l	16	176.2±88.9
Parasite count/ml	16	5657.08±2485.3

The bone marrow intracellular iron was assessed only in nine patients. There was no intracellular iron in two (22%) patients scanty in 2(22%), nonnal in2 (22%) and increased in 3 (34%).

## **Discussion**

The present study reports the bone marrow changes in malaria in North West Frontier Province of Pakistan, where malaria is endemic and to compare it with other studies done in other parts of the World. Vivax malaria was not associated with significant pathology except mild megaloblastic erythropoiesis in one (11%) case. The single (4%) patient with mixed infection had hypercellular marrow and megaloblastic erythropoiesis.

Out of 26 bone marrows recorded during 39 months period, 16 (61%) showed the presence of plasmodium falciparum. In 50% the bone marrow was normocellular, in 31% hypercellular and in 12% hypocellular. All the patients with hypercellular marrow belonged to severely anaemic group 2. The bone marrows of 20 Gambian children showed varying degrees of hypercellularity which was more marked in severely anaemic patients. The hypercellularity was due to erythroid hyperplasia in patients with chronic malaria<sup>3</sup>. In our patients, the hypercellularity in severely anaemic patients, was both due to erythroid myeloid hyperplasia.

In another study of seven Gambian children suffering from falciparum malaria, bone marrow had severe erythroid hyperplasia and dyserythropoiesis. One (14%) each had megaloblastic and macronormoblastic and 5 (71%) normoblastic erythropoiesis<sup>4</sup>. Three (18%) patients in this series had erythroid and myeloid hyperplasia. Fleming<sup>5</sup> examined the bone marrows of 34 pregnant patients suffering from falciparum malaria in Ndolla (Zambia) and reported erythroid hyperplasia in 26(76%), myeloid hyperplasia in 25 (74%), excess eosinophil precursors in 3 (9%), increased lymphocytes in 3 (9%) and normal plasma cells in 11(38%) cases. He reported definite megaloblastic erythropoiesis and myelopoiesis in 23 (68%) bone marrows. He observed dyserythropoiesis in only one (3%) bone marrow. Anaemia associated with malaria and megaloblastic erythropoiesis in his series was characterized by younger age of the patients, greater severity of anaemia, macrocytosis, erythroid hyperplasia and neutrophil leucocytosis.

The findings in this study are similar to those reported by Fleming<sup>5</sup> except the presence of dyserythropoiesis in one case, which was not found in any patient in our series, although he studied pregnant patients with falciparum malaria. In our patients with megaloblastic changes both red cell folate and serum vitamin B<sub>12</sub> level were normal. Malarial parasite produces glycosyl phosphatidyl

inositol (GPI), a novel class of glycolipid toxins, which substitute for the endogenous inositolglycan based signal transduction pathways of the host. GPI stimulates high levels of tumor necrosis factor (TNF) and interleukin 1 produced by macrophages. These mediators are responsible for the production of these morphological abnormalities and anaemia<sup>6</sup>. Elshoura<sup>7</sup> reported several ultra structural changes in non-parasitized erythroblasts in the bone marrow and erythrocytes in the peripheral blood of 28 Saudi patients who were anaemic and suffering from acute falciparum malaria. The erythroblasts for the first time showed conspicuous surface knobs which were previously described only for the parasitized erythrocytes. Ultrastructural changes of these cells were suggested to be due to dyserythropoiesis, which were in turn attributed to an imbalance in metabolism as they were being overproduced in response to infection. Numerous haemoglobin like particles were being liberated through the erythrocyte plasma membrane indicating severe haemolysis which is considered to be one of the major factors producing anaemia during malarial infection. Jootar and coworkers<sup>8</sup> cultured the bone marrows of 21 Thai adults infected with *Plasmodium falciparum* for CFU and BFU using AB serum, autologous serum (parasitaemia) and autologous serum (post parasitaemia). Fifteen patients had haematological complications. The autologous sera during parasitaemia suppressed the growth of CFU-E and BFU-E, both during and after parasitaemia. They postulated that two possible mechanisms for this suppression are the reduction of erythropoietin or the increased TNF production during malarial infection. This retrospective study shows that bone marrow involvement by the *Plasmodium falciparum* does occur in Pakistan where malaria is endemic. The changes in the bone marrow particularly in the red blood cells are not so marked as reported by workers from other countries<sup>3-8</sup>. Why *Plasmodium falciparum* produces dyserythropoiesis in some infected individuals but not in others, as in our situation is poorly understood. This may be due to the polymorphism of *Plasmodium falciparum* isolates in inducing different levels of TNF which may differ by 100 folds as demonstrated by Allan and coworkers<sup>12</sup>. Again TNF production may also be determined by the variation in the propensity of the host to produce TNF, the variation in the propensity of the host to produce TNF, the population dynamics of the parasites within the host and the acquisition of antitoxic antibodies and other immune adaptations of the host.

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