Plasmodia in bone marrow examinations: Case series from a hospital in Pakistan 2007-2015

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Research Article

Keywords: malaria, Pakistan, reticuloendothelial, bone marrow, plasmodia

Posted Date: April 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-418977/v1>

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Abstract

Background Malaria is a life-threatening, multisystem disease caused by the protozoan plasmodia with a global incidence of approximately 212 million annually. The parasite is known to have unique and crucial interactions with various body tissues during its life cycle, notably the liver, spleen, and recent work has shown the bone marrow as reservoir of infection.

Methods This study is a case series of patients in whom examination of bone marrow revealed malaria parasites. We conducted a retrospective record review of 35 malaria-positive bone marrow specimens examined at Aga Khan University Hospital (AKUH), Karachi, Pakistan, over the years 2007 to 2015. Bone marrow aspirates were collected as per International Council for Standardization in Haematology (ICSH) guidelines.

Results The median age of patients was 22 years (range 1–75), and 60% (n = 21) were male. 22 patients had evidence of *P. falciparum*, 12 had evidence of *P. vivax* and 1 patient had a mixed infection. Gametocytes and trophozoites were the most common stages identified on both peripheral blood and bone marrow examinations. Indications for bone marrow examination included fever of unknown origin and the workup of cytopenias and malignancies.

Conclusions The incidental finding of plasmodia in samples of bone marrow from our patients suggests the reticuloendothelial system may be regularly infected by these parasites, be it acute or chronic in character.

Introduction

Acute malaria is a life-threatening, multisystem disease caused by protozoan plasmodia that infect hundreds of millions annually, with several hundred thousand not surviving [1]. These parasites occur as multiple forms in varied tissues, including liver, blood spleen, and bone marrow [2]. *Plasmodium vivax*, in particular, exhibits specific molecular traits that would appear to accord with strong tropisms for hemopoietic tissues [3]. Merozoites of that species require transferrin receptor 1 (CD71) to invade erythrocytes [4], a molecule that vanishes from the surface of that cell during early reticulocyte development [5]. The parasite would thus appear to have almost no opportunity to reproduce in peripheral blood, but would have almost limitless access to cells bearing that receptor in the extravascular spaces of hemopoietic tissues. Indeed recent work with *P. vivax* in splenectomised rhesus macaques demonstrated those spaces of bone marrow as the dominant compartment of that infection [6]. *Plasmodium falciparum* has also been shown to interact with bone marrow; employing it as a site of maturation and for evasion of the host immune response [7–12]. The anaemia that often develops with acute malaria involves bone marrow suppression and ineffective erythropoiesis [13–16].

Sample of bone marrow are obtained by an invasive procedure and collected when clinically indicated by suspicion of specific illnesses, or at autopsy. Malarial parasites were first demonstrated in the bone marrow on autopsies of lethal malaria cases in 1892, not long after the discovery of the malarial parasite [3]. Later, bone marrow aspiration was described as an accessory and useful tool in the diagnosis of malaria especially in cases where strong suspicion of malaria could not be confirmed on routine peripheral smears [8–10]. Peripheral smear examination under light microscopy remains the gold standard of malaria diagnosis and consistently negative findings in a patient are considered definitive. Point-of-care rapid diagnostic tests (RDTs) on peripheral blood are also widely used. However, malaria in endemic zones is often dominated by sub-patent and typically asymptomatic infections [17]. The finding of plasmodia in samples of bone marrow in endemic settings may thus occur with negative findings from peripheral blood [18–20].

Currently, bone marrow trephine biopsy and aspiration are routinely done for the workup of various haematological, infiltrative and storage abnormalities [21, 22]. In general, malaria infection of bone marrow is usually an incidental finding to those investigations. It is not clear if undiagnosed malaria explained the illnesses prompting bone marrow

examination, or if it was clinically silent and of no direct relationship to the presenting illness. The findings on bone marrow of patients with malaria range from normocellular, normoblastic erythropoiesis to hypercellular, dyserythropoietic erythropoiesis [23–25]. The current study adds to that scant body of evidence by reviewing the finding of malaria infection incidental to bone marrow examination at our teaching hospital in Karachi, Pakistan.

Methods And Patients

We conducted a retrospective record review of all malaria-positive bone marrow specimens examined at Aga Khan University Hospital (AKUH), a large tertiary care hospital in Karachi, Pakistan, from the years 2007 to 2015. Endemic malaria transmission occurs seasonally in and around Karachi, and across much of Pakistan.

Bone marrow aspiration was either done at Aga Khan University, Main Campus or submitted for review at one of our collection points across Pakistan. A complete blood cell count and peripheral blood smear was also routinely done at the time of bone marrow aspiration. In all cases, bone marrow aspirate and trephine biopsy were collected from posterior superior iliac spine. Bone marrow aspirates were collected as per International Council for Standardization in Haematology (ICSH) guidelines. Ten to 20 ml plastic syringes, without anticoagulant, were used to draw out the aspirate. The first 0.5ml were used to produce smears at bedside while the remainder was put in a tube containing a proportional amount of di-potassium ethylenediaminetetraacetic acid (EDTA) to prevent sample coagulation. Bone marrow slides were prepared immediately after aspiration. The slides were stained with Leishman stain for microscopy.

Core biopsies of bone were taken using Jamshidi needle. Whenever, possible 2cm long core was obtained in each case. Specimens were transported and fixed in 10% buffered formalin. After decalcification in 10% formic acid, paraffin-wax embedding was done. Sections, 4-µm-thick, were cut by experienced histotechnologists using standard equipment and used for hematoxylin and eosin staining.

Patients included in the study were either already diagnosed with malaria and their bone marrow was examined for work-up of another condition, or the finding of malaria pigment/parasite was incidentally discovered in the bone marrow sample.

Peripheral blood smear was considered positive for malaria when any stage of malaria life cycle was identified. In addition, bone marrow aspirate was considered positive for malaria when any stage of malaria life cycle or malaria hemozoin pigment was identified.

Following exemption from the ethics review committee (3672-Pat-ERC-15), anonymized data was collected from past records and analysed using Statistical Package for the Social Sciences (IBM SPSS) version 19. The data reviewed for each patient included: a) patient demographics, b) relevant, significant findings on history and physical examination, c) a complete blood count and blood peripheral film of a blood sample taken at the time of bone marrow sampling and d) final report of bone marrow aspirate and/or trephine biopsy done on bone marrow samples.

Frequencies and percentages were calculated for categorical variables and mean and standard deviation for continuous variables. For statistical analysis, Fisher's exact test was used for categorical variables whereas Mann-Witney U was employed for continuous data.

Results

A total of 25,867 bone marrow samples were received in our laboratory during the study period. Thirty-five (0.13%) patients were reported as positive for malaria parasites. The median patient age was 22 years (range 1–75), comprising of 60% (n = 21) male patients. Fever and cytopenias were the two most common indications for bone marrow examination, accounting for approximately two thirds of cases (Table 1). Hepato/splenomegaly was the next most

common indication. All the patients were symptomatic and the most common symptoms reported were fever and fatigue (Table 2). *Plasmodium falciparum* was identified in 22 patients, whereas, 12 patients had *P. vivax* (Figs. 1 & 2), and one patient had a mixed species infection. Table 3 lists the various stages of plasmodia identified on blood and bone marrow. Four patients were positive by marrow examination but not peripheral blood smear. Three of these had *P. falciparum* infection and a fourth patient had a mixed infection. In another patient with *P. vivax* on peripheral blood, hemozoin pigment was the only evidence of malarial infection of the bone marrow (Table 3).

Table 1
Indications for bone marrow examination in cases later found to be positive for malaria.

Indication of Bone Marrow Biopsy		N (%)
Fever		13 (37.1)
Cytopenias	Anemia	1 (2.8)
	Thrombocytopenia	3 (8.6)
	Bi or pancytopenia	8 (22.8)
Hepato/splenomegaly		7 (20)
Staging Hodgkin's lymphoma		1 (2.8)
Staging of Neuroblastoma		1 (2.8)
For remission status of Leukemia		1 (2.8)

Table 2
Clinical features of the case series.

Symptoms and signs reported	n (%)
Fever	22 (62.85%)
Pallor	17 (48.57%)
Fatigue	18 (51.42%)
Weight loss	14 (40%)

Table 3

Various Plasmodia stages and hemozoin pigment detected in peripheral blood (PB) and Bone marrow (BM).

Species	Trophozoites on PB	Gametocytes on PB	Schizonts on PB	Trophozoites on BM	Gametocytes on BM	Schizonts on BM	Hemozoin pigment in BM
<i>P. vivax</i> (n = 11)	10* (83.3)	10* (83.3)	2* (16.7)	9 (75.0)	9 (75.0)	2 (16.7)	4 (33.3)
<i>P. falciparum</i> (n = 22)	11 (50.0)	11 (50.0)	-	12 (54.5)	15 (68.2)	-	11 (50.0)
Mixed (n = 1)	1 (100.0)	1 (100.0)	-	1 (100.0)	1 (100.0)	-	1 (100.0)
* For one patient a peripheral blood sample was not available							

Table 4

Complete blood count parameters in patients with *P. vivax* and *P. falciparum*

Haematological Parameters	<i>Plasmodium vivax</i>	<i>Plasmodium falciparum</i>	P-value
Hb (g/dl)	9.77 (± 1.44)	7.73 (± 1.72)	0.003*
Hematocrit	30.17 (± 4.73)	23.17 (± 4.81)	0.001*
Platelet Count	74.45 (± 74.82)	81.5 (± 67.49)	0.801
WBC	5.9 (± 2.84)	5.18 (± 3.22)	0.549
ANC	3 (± 2.19)	2.05 (± 0.75)	0.18
MCV	80.9 (± 4.8)	85.3 (± 10.6)	0.206
MCH	26.26 (± 2.11)	28.29 (± 3.23)	0.049*

Discussion

In Pakistan the two most prevalent causes of malaria are *Plasmodium vivax* and *Plasmodium falciparum* constituting 64% and 36% cases, respectively [26]. In contrast, most of the infections in our case series was *P. falciparum* (63%). We do not believe that the bone marrow samples were taken on suspicion or confirmation of malaria. Malaria may be viewed as an incidental finding in bone marrow examination undertaken for various other indications (Table 1). However, since, most samples were received outside our centre, we did not know the result of microscopic examination of peripheral blood film routinely done prior to ordering the bone marrow procedure. Ideally, bone marrow examination would not have been performed in case where peripheral blood smear was positive for malaria parasite. Only 4 of our 35 patients had no evidence of plasmodia on peripheral film. Whether the parasite identification was missed on the initial peripheral smear, or it was absent in early peripheral smears cannot be confirmed.

The primary cause of illness among our 35 patients appeared to be consistent with chronic low-grade malaria infection. Most of them were markedly anemic (mean of 8.6g/dL) and they recovered with antimalarial therapy. So-called "asymptomatic malaria" of chronic, low-grade parasitemias are indeed associated with states of anemia [27].

Pathologists examining bone marrow should be alert to the possibility of malaria as the cause of illness prompting that examination.

It may be likely that most cases of malaria in our catchment area were indeed detected prior to initiating the bone marrow sample, as suggested by the low frequency of malaria (0.13%) among the 25,867 bone marrow examinations we evaluated. We cannot count the number of bone marrow examinations averted by an early diagnosis of malaria on peripheral blood smear, but it may be considerably larger than the cases we describe here. At least in the small sample of bone marrow samples found positive for malaria, the even rarer numbers lacking parasites on peripheral blood smear suggests that infection of bone marrow without parasitaemia may be the exception. A systematic review of infections among blood donors during 2010 to 2020 in Pakistan found 0.11% positive for malaria [28]. We cannot know if infection of blood by the plasmodia is usually attended by infection bone marrow, the similarities of those rates of infection of blood and bone marrow suggests that may be the case.

Conclusions

Our case series of 35 patients found to have malaria presented with illnesses that prompted bone marrow examinations, and they represented a small minority of patients undergoing that procedure. Infection of bone marrow may be a common feature of malaria, be it acute or chronic in character.

Declarations

Ethics approval and consent to participate: This study was approved by Aga Khan University ethical review committee (AKU-ERC).

Consent for publication: Not Applicable

Availability of data and materials: The datasets used and analysed during the current study are available from the corresponding author on reasonable request. Most of data analysed during this study are presented in this published article.

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Authors' contributions: MSS and MAB designed and planned the study. BA, MJ, AA and SAH contributed to data collection and writing of initial draft of manuscript. MSS, MAB and JKB extensively reviewed the manuscript. BM contributed to conception of the study and reviewed the manuscript critically. AR analyzed the data and contributed to writing of results. All authors read and approved the final manuscript.

Acknowledgements: Authors are thankful to laboratory staff who helped in retrieval of archived bone marrow cases.

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Figures

Figure 1

Malaria parasite *Plasmodium vivax*, trophozoites and schizont in various stages of development within a sample aspirated from the bone marrow.

Figure 2

Malaria parasite *Plasmodium vivax*, trophozoites, gametes and schizont in various stages of development within as sample aspirated from the bone marrow.