Pancytopenia induced by secondary hemophagocytic lymphohistiocytosis: A rare, overlooked dreadful complication of *Plasmodium vivax*

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Abstract

Hemophagocytic lymphohistiocytosis (HLH) is an unusual multifaceted clinicopathological entity that often remains misdiagnosed and can be fatal if not timely detected or treated. It can be familial or associated with different types of infections, autoimmune disorders, and malignancies. Parasitic infection-associated HLH has been rarely documented in the literature with only a handful of them being reported due to *Plasmodium vivax* infection. We describe an extremely rare case of pancytopenia induced by HLH resulting from *P. vivax* infection in a 7-year-old girl, which posed as a diagnostic challenge and led to a therapeutic delay.

Keywords: Bone marrow biopsy, hemophagocytic lymphohistiocytosis, malaria, pancytopenia, *Plasmodium vivax*

INTRODUCTION

Malaria is a global as well as a national public health concern in several countries. This devastating disease is widespread in the tropical and the subtropical regions including much of Sub-Saharan Africa, Asia, and Latin America. According to the latest World Health Organization (WHO) estimates, there were 219 million cases of malaria in 2017, up from 216 million cases in 2016. In India, malaria is a major socioeconomic burden, as this country not only has the third-highest number of cases in the world but also accounts for the highest malarial load of 75% in WHO Southeast Asia Region. It is particularly entrenched in rural, tribal, hilly, hard-to-reach, or inaccessible areas of the central, eastern, and north eastern states of India.

The protozoan parasite *Plasmodium* is the causative agent of this life-threatening condition and it is Anopheles mosquito-borne infectious disease. Among the various *Plasmodium* species infecting the humans, *Plasmodium falciparum* is the most virulent and is associated with severe complications and eventually death if left untreated. Other species such as *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi* generally cause a milder form of malaria and rarely any complications. However, recent evidences have suggested that *P. vivax* malaria is associated with certain lethal conditions about as often as seen with *P. falciparum* infection, though they have been often overlooked and under-reported. We herein illustrate one such rare potentially fatal complication of hemophagocytic lymphohistiocytosis (HLH) developing...
secondary to \textit{P. vivax} infection, which induced pancytopenia in a 7-year-old girl and created a diagnostic conundrum as well as therapeutic deferment.

\section*{CASE REPORT}

We received a bone marrow biopsy measuring 2 cm in length in the Hematology Unit of the Department of Pathology along with the requisition form mentioning the following clinical details: 7-year-old girl, resident of New Delhi, India, and clinical features – fever on and off associated with weakness and abdominal distension for the past 3 weeks. Physical examination revealed pallor and hepatosplenomegaly with no significant lymphadenopathy. Laboratory data showed pancytopenia with a hemoglobin level of 8.7 g/dl, the total leukocyte count of 3400/mm$^3$, and a platelet count of 44,000/mm$^3$. Peripheral smear showed microcytic hypochromic blood picture. Bone marrow aspiration finding reported from an outside center was of hypocellular marrow. Human immunodeficiency virus serology, hepatitis B surface antigen, antibodies against hepatitis C virus, the Epstein–Barr virus, cytomegalovirus, measles, Widal test, K39 test for visceral leishmaniasis, and rapid diagnostic tests (RDTs) for malarial antigen were negative. Her blood, urine, sputum, and stool cultures were sterile. The chest X-ray and the cerebrospinal fluid study were normal.

The bone marrow biopsy that we received was routinely histoprocessed and stained with hematoxylin and eosin stain. Low power microscopic examination of the sections revealed an adequate bone marrow in length with normal bony trabeculae. The marrow was normocellular for the age [Figure 1a]. High-power view of the trephine biopsy exhibited sheets and clusters of histiocytes. These were large cells with abundant pale staining bubbly cytoplasm and a small nucleus. No pleomorphism or any prominent nucleoli were seen. These histiocytes were seen admixed with an increased number of erythroid precursors and lymphocytes. The rest of the marrow hematopoietic elements were suppressed [Figure 1b]. Many of these marrow macrophages demonstrated phagocytosis of red cells, white cells, platelets as well as immature myeloid and erythroid cells (hemophagocytosis) [Figure 2a]. Some of these histiocytes also revealed the presence of a brown-colored pigment [Figure 2b]. Based on these findings, a tentative diagnosis of a mononuclear phagocytic system disorder/histiocytosis was made. Special stains and immunohistochemistry (IHC) were applied for ruling out various differentials under this category of disorder such as storage histiocytosis, dendritic cell-related, inflammatory, and malignant histiocytosis. On IHC, the histiocytes were positive for CD68 and negative for CD1a, S100, CD3, CD20, epithelial membrane antigen, and pan-cytokeratin, confirming the histiocytic nature of these large cells and ruling out the possibility of dendritic cell-related (Langerhans cell histiocytosis) and malignant histiocytosis [Figure 3a–c]. However, on IHC, a finding that was evident was the CD3 positive immunoreexpression of the increased lymphocytes [Figure 3d]. Among the special stains, the periodic acid–Schiff (PAS) stain, Oil red O stain, and Perl’s iron stain were put. These histiocytes were negative for both the PAS stain as well as the Oil red O stain.
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Figure 4: (a) Periodic acid–Schiff stain negative histiocytes (PAS, ×200). (b) Perl’s Prussian blue staining showing unstained brown pigment in the histiocytes (Perl’s stain, ×200)

Figure 5: (a) Schizont of *P. vivax* (encircled) on peripheral blood smear examination (Leishman stain, ×200). (b) Bleaching of the brown-colored pigment in the histiocytes with the alcoholic ammonium hydroxide (H and E, ×200)

Pancytopenia was treated with alcoholic ammonium hydroxide, to which the brown pigment responded by getting bleached, hence, confirming the nature of this pigment to be that of malarial pigment (hemozoin) [Figure 5b]. Based on the clinical, hematological, histopathological, and immunohistochemical parameters, a final diagnosis of pancytopenia due to HLH as a result of *P. vivax* infection was established. Due to the cost and unavailability, local perforin mutation studies and soluble interleukin (IL) level estimation tests were not performed. The child was administered oral chloroquine and primaquine along with iron supplements. Her condition dramatically improved within few days as she became afebrile, her laboratory parameters such as serum ferritin and fibrinogen levels improved gradually, and the liver and spleen reduced in size. She was discharged in a stable condition (hemoglobin of 10.8 g/dL, total leukocyte count of 10,600/mm³, and a platelet count of 198,000/mm³), and so far, her follow-up period has been uneventful.

DISCUSSION

Malaria is frequently associated with innumerable hematological complications which, in turn, contribute to the morbidity and mortality of this disease. Two unusual interlinked manifestations of malaria are pancytopenia and HLH, which have been mainly reported with *P. falciparum*.[8,9] Pancytopenia due to *P. vivax* malaria is extremely rare and so far has been documented in 0.9% of confirmed *P. vivax* cases.[10] It may cause pancytopenia via HLH, myelosuppression, hypersplenism, or tumor lysis by infection-related steroid release.[11] The understanding of this interconnection between the HLH and *P. vivax* is of great importance as it has direct diagnostic and therapeutic implications.

Pancytopenia is a condition which is encountered daily in routine clinical practice. Nevertheless, it is not a disease entity but a triad of findings that may result from a
number of disease processes such as megaloblastic and aplastic anemia, hematologic malignancies, metastatic cancer, infection, and or inflammation. These disorders may affect bone marrow either primarily or secondarily and result in manifestations of pancytopenia which makes the patient prone to anemic symptomatology, infections, and hemorrhagic diathesis.\[^{12}\] HLH is a rare cause of pancytopenia and is an uncommon life-threatening disorder which was first described by Scott and Robb-Smith in 1939 as histiocytic medullary reticulosis.\[^{13}\] It is characterized by reactive, systemic proliferation of benign histiocytes throughout the reticuloendothelial system. However, its incidence is not well reported as this entity is often misdiagnosed. It exists in two main forms: primary/familial or secondary. Familial HLH is usually diagnosed at an early age, while the onset of secondary HLH is independent of age and occurs due to infections, autoimmune diseases, and malignancies. Among various infections, viral infections mainly cause HLH. Nevertheless, rarely, secondary HLH cases can also occur due to bacterial, parasitic, fungal, and protozoal infections.\[^{14}\] The probable pathogenesis behind it is inappropriate or excessive immunological response of natural killer and cytotoxic T-cells leading to a cytokine storm due to the elaboration of cytokines such as IL-1, IL-2, IL-6, tumor necrosis factor-\(\alpha\), interferon-\(\gamma\), and macrophage colony-stimulating factor (M-CSF) by the T-helper cells which promote activation of macrophages resulting in phagocytosis of the hematopoietic cells, marrow suppression, and disturbance in clotting cascade.\[^{15}\]

\(P. \text{ vivax}\) is a very rare cause of pancytopenia associated with HLH. This association between pancytopenia and HLH has been explored intensively by various researchers who have observed strikingly high levels of these cytokines in patients with malaria which can possibly trigger HLH initiation.\[^{16,17}\]

After extensive research of the pertinent world literature, only a handful of cases secondary to \(P. \text{ vivax}\) associated pancytopenia induced by HLH have been reported so far.\[^{10,14,15,18-28}\] On further exploring these cases mentioned till date, various clinicopathological features of this association warrant the attention. Most of the cases were restricted to the malaria endemic regions. Prolonged fever, hepatosplenomegaly, anemia, leukopenia, thrombocytopenia, hyperferritinemia, detection of trophozoites and gametocytes of \(P. \text{ vivax}\) on peripheral smear, and hemophagocytosis on bone marrow examination were the most common clinical, biochemical, and pathological findings. Maximum number of the patients recovered without any complications with antimalarial treatment.

In the present case study, although the majority of the features of the case were consistent with the previously documented worldwide cases, there were many confounding factors leading to a diagnostic dilemma and therapeutic delay in this case. First, initially, the RDTs for the malarial antigen were false-negative, and the \(P. \text{ vivax}\) was also missed on the peripheral blood smear examination, leading to bone marrow biopsy for evaluating the cause of pancytopenia. Second, even after thorough bone marrow biopsy examination and making a differential diagnosis with the help of IHC and special stains, the final diagnosis was made only after clinching the \(P. \text{ vivax}\) schizont on carefully examining the repeat blood sample for the peripheral smear. Nevertheless, these unusual scenarios have been documented and well-supported in the literature by few researchers who have mentioned that there are false-negative RDTs results, and very rarely, the parasites are not found in the peripheral blood smears from the patients of malaria, even in severe infections; therefore, in such a setting, these cases are usually diagnosed based on bone marrow study alone.\[^{29,30}\]

False-negative RDTs results are far more likely to be due to the procurement and use of poor quality RDTs or use of the wrong comparator for the diagnostic test, such as low quality microscopy for cross-checking negative RDTs results.\[^{13}\] Poor transport and storage conditions for RDTs, with sustained exposure to high temperature, can affect their diagnostic performance and operator errors during performance, and/or interpretation of RDTs results can also give false-negative results. Other important factors are the parasite components such as the lack or low-level expression of the target antigen of the parasite or if there is any variation in the amino acid sequence of the epitope targeted by the monoclonal antibody. Host–parasite density such as very low parasite density or target antigen concentration as well as very high parasite load (severe malaria) causing prozone effect also plays a pivotal role in its detection by RDTs. On the other hand, the peripheral blood smear misinterpretation may be attributed to the pretreatment with antimalarial drugs in inadequate doses, causing partial clearance of the parasite, low levels of parasitemia not detected by conventional microscopy or by sequestration of the parasitized cells, in deep vascular beds.\[^{19}\]

Various authors have also mentioned that it is difficult to determine the prevalence of malaria complicated by HLH as bone marrow examination is usually not done for the purpose of diagnosing malarial infection.\[^{19}\] However, if by chance the bone marrow studies are undertaken as in the atypical case of ours, erythrophagocytosis and malaria parasite is usually observed in the bone marrow of the patients with malaria.\[^{22}\] In our patient, bone marrow biopsy showed histiocytes containing erythrocytes, leukocytes, platelets, immature myeloid and erythroid cells along with...
abundant hemozoin pigment in some histiocytes which was confused with hemosiderin initially and was only confirmed as hemozoin after getting a clue on observing the schizont of P. vivax on the repeated peripheral blood smear examination, following which the biopsy was treated with alcoholic ammonium hydroxide. Overall, the present case fulfilled the current diagnostic criteria of HLH; however, one should be aware of the fact that the absence of phagocytosis on bone marrow does not rule out the diagnosis of hemophagocytic syndrome. Several other tests such as estimation of serum cytokines, in vitro activity test for erythrophagocytosis, mutational studies, and species confirmation by polymerase chain reaction also aid in its diagnosis and help in differentiating primary and secondary cases. However, these investigations are usually not done in routine clinical practice, as most of the centers, especially in developing countries like India, lack molecular studies, and even if they do have the facilities, the patients are unable to afford them due to financial limitations.

The patients with infection-associated HLH usually recover with supportive care and with the treatment being targeted to the underlying infection in such cases. In the present case too, chloroquine was given once P. vivax was detected, and the patient outcome was favorable. This finding has been well-supported by many authors who have mentioned in their studies that the malaria-induced HLH usually responds to antimalarial drugs alone; however, rarely, steroids have also been used as a treatment. The treatment response of such patients is assessed by the resolution of clinical signs/symptoms and laboratory findings such as serum ferritin levels, liver function tests, and fibrinogen levels.

In our patient, serum ferritin as well as fibrinogen levels and other hematological parameters improved significantly after treatment, along with the clinical resolution of the disease.

The mortality rate due to HLH is although not well-established, nevertheless, secondary HLH is fatal in the absence of treatment. Various authors have documented that hemophagocytosis is one of the complications of hemophagocytic syndrome in malaria and results in prolonged anemia. However, till date, this has been reported in P. falciparum malaria and not yet in P. vivax malaria. Other complications observed are hyperbilirubinemia, acute renal failure, encephalopathy, seizures, and coagulation abnormalities.

**CONCLUSION**

This case underscores the association of P. vivax with HLH and the crucial role played by HLH in the pathogenesis of pancytopenia observed during Plasmodium infestation. A high index of clinical suspicion should be envisaged, especially in people residing in or travelled from malaria endemic areas. Early detection of parasite with the use of good quality RDTs and vigilant as well as repeated peripheral blood smear examination is paramount for timely diagnosing such a rare amalgamation to prevent any irreversible end-organ damage and to avoid an invasive procedure such as bone marrow aspiration/biopsy since these cases respond quickly to antimalarial drugs and have a good prognosis.

**Declarartion of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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