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Case Report

A Case of Mixed *Plasmodium falciparum* and *Plasmodium malariae* Infection in a 1 Year 04 Months Old Child in Western Region, Côte d`Ivoire

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Abstract

Malaria is the most common vector-borne parasite infection globally, resulting from the infection by one of the five Plasmodium species identified thus far in human. Approximately 80%of malaria cases are caused by *P. falciparum*, in West Africa and other tropical to temperate regions. *P. malariae* and *P. ovale* are responsible for a relatively small proportion of malaria casesin Africa. Here, we report the case of a 1 year 4 months old child (Ms. Z.P) who acquired a mixed *P. falciparum* and *P. malariae* infection. She was diagnosed with *P. falciparum* and *P. malariae* infection on the basis of peripheral blood smear (PBS). She was treated with Novalgin 0,2 cc in IM and Artemether 23 mg in IM. We started a rescue therapy with 140 ml of a whole blood groupO Rhesus positive, and the patient recovered successfully. This is an important finding suggestinga comprehensible check on children with negative RDT test in malaria endemic countries.

Introduction

Parasitic infections such as malaria are common under the tropics. They lead to high morbidity and mortality, especially in children. In 2020, approximately 241 million people were infected with malaria and an estimated 627 000 of them died worldwide of which 95% of malariadiagnosed cases and 96% of the global deaths were attributed to the World Health Organization (WHO) African Region [1]. Malaria is responsible for acute or chronic anemia that is globally estimated in 2020 at 39.8% in children aged 6 to 59 months and equates to 269 million anemic children [1].

In Côte d'Ivoire, the prevalence of malaria in 2017 was estimated at 49% and 52%% on the basis of the Giemsa-stain thick blood smear and the rapid diagnostic test (RDT), respectively [2]. The prevalence of anemia in 2019 among children aged 6 to 59 months was 72.2%[3]. In most cases, the poly-parasitism is the rule and these conditions affectthe same people, causing morbidities that vary according to age and region [4].

Here, we report a case of malaria with severe anemia that was caused by a mixed *P. falciparum* and *P. malariae* infection and conclusively diagnosed with a peripheral blood smear (PBS) even

though RDT testing from 3 different manufacturers were negative.

Case Description

Clinical history and laboratory findings

A 1 year 04 month-old female child (Ms. Z.P), Burkinabe of origin residing in Côte d`Ivoire (Figure 1), who consented two day ago and was screened for participation in an anemia and parasitic infestations study on 20/Jan/2021 (Study was approved by the "Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS) de la Côte d'Ivoire" (N/Ref: 024- 21/MSHP/CNESVS-km). She was rushed to the Regional Hospital Center of Man (CHR Man) on22/Jan/2021 for severe anemia (hemoglobin (Hb) rate at 4.7 g/dl).

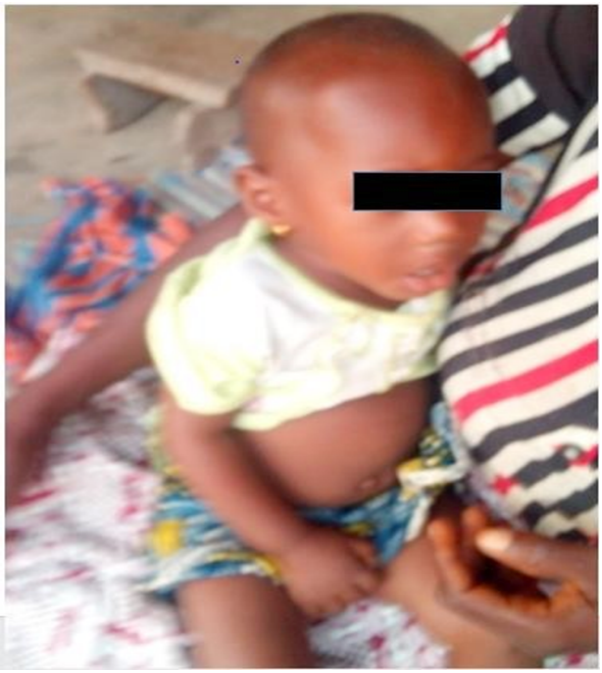

Figure 1: Ms. P.Z on the laps of her mother.

The participant had been in her usual state of health until she consented in an anemia study a day before her admission for blood transfusion. There was no history of consultation. However, the mother reported to the study team during a late home visit on the day of her admission to the research study after the laboratory results were released (to ascertain the child health status - clinical decompensation) that for sometimes her child has been on and off for unwellness.

Once at the regional hospital (CHR Man) the following morning, the participant was rushed to the Paediatric services for evaluation. On examination, the participant weight was 7.6 kilograms, febrile with a temperature at 38.2°C, the blood pressure 100/60 mm Hg, the pulse at 112 beats per minute, the respiratory rate at 28 breaths per minute and stable. The overall clinical condition was normal, the conjunctiva were pales, the abdomen flexible, non-tender without a palpable mass. The lungs were free and no oedema of the inferior members was observed. A suspicion of severe malaria diagnosis was made, Novalgin 0, 2 cc in IM and Artemether 23 mg in IM for 3 days were initiated, and the patient was admitted.

In view of the screening results, a control FBC and a blood group & Rhesus group were requested together with a Giemsa thick and thin blood smear.The malaria rapid diagnostic test (governmental free test for plasmodium falciparum species only) performed was negative. Prior to that, during the screening period in her village two (2) others rapid diagnostic test for malaria from two different manufacturers [A = Humasis Co. Ltd. (malaria *P.f*/ Pan Antigen Test, Humasis, South Korea), LOT No.: MAL9002 and B = Hangzhou All Test Biotech Co., Ltd., China. LOT No.: MAL 2011437-S) were performed and the results were both negative (Figure 2). These commercialized tests were intended to detect all four (4) species (*P. falciparum, P. malariae, P. ovale* and P. *vivax*). Additionally, some screening testsperformed on behalf of the study such as urine analysis, urine filtration, POC-CCA, Kato-Katz forthe diagnostic of *S. haematobium* and *S. mansoni* were all negatives.

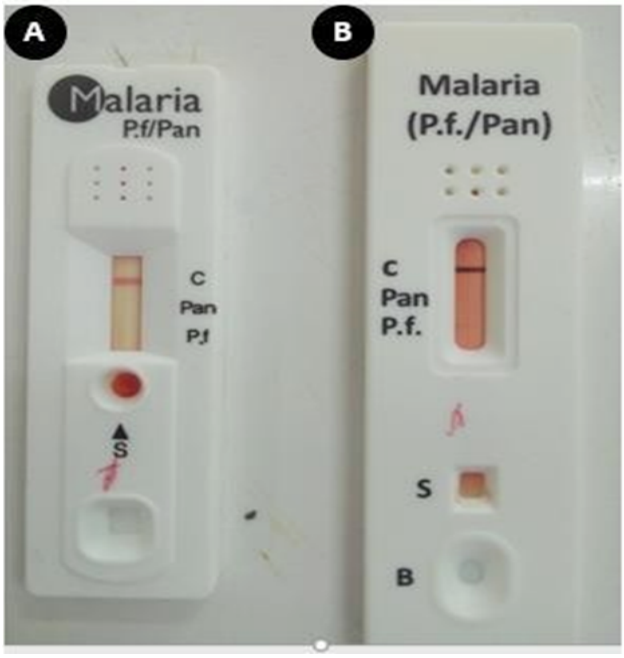

Figure 2: RDT results from manufacturers A and B.

The initial hematology results during screening (Table 1) and the released control results (Table 2) as well as the microscopy Giemsa- thick and thin performed results are shown in figure3.

Test	Result	Reference range	Unit	Alert
White blood cells	4.90	4.50-13.00	$x10^3$ /mm^3	
Red bloodcells	3.50	4.80-5.50	$X10^6$ /mm^3	Low
Hemoglobin	4.70	12.00-16.00	g/dl	Low
Hematocrit	23.0	37.0-47.0	%	Low
MCV	64.70	85.00-95.00	fl	Low
MCH	18.50	27.00-31.00	pg	Low
MCHC	27.60	32.00-36.00	g/dl	Low
Platelet	177.5	160.00-400.00	$x10^3$ /mm^3	
PNN %	46.2	45.0-70.0	%	
PNN (Abs)	2300.00	1500.00-7000.00	/mm^3	
Lymp %	42.6	20.0-40.0	%	High
Lymp (Abs)	2100.00	1500.00-4000.00	/mm^3	
Mono %	11.4	2.0-10.0	%	High
Mono (Abs)	500.00	0.00-1000.00	/mm^3	
PNN = Ploymorphonuclear neutrophils, Lymp = Lymphocytes, Mono = Monocytes				

Table 1: Initial FBC performed during the screening process.

Test	Result	Reference range	Unit	Alert
White blood cells	5.00	4.50-13.00	$x10^3$ /mm^3	
Red bloodcells	3.57	4.80-5.50	$X10^6$ /mm^3	Low
Hemoglobin	6.60	12.00-16.00	g/dl	Low
Hematocrit	23.1	37.0-47.0	%	Low
MCV	64.70	85.00-95.00	fl	Low
MCH	18.50	27.00-31.00	pg	Low
MCHC	27.60	32.00-36.00	g/dl	Low
Platelet	177.0	160.00-400.00	$x10^3$ /mm^3	
PNN %	46.2	45.0-70.0	%	
PNN (Abs)	2300.00	1500.00-7000.00	/mm^3	
Lymp %	42.4	20.0-40.0	%	High
Lymp (Abs)	2100.00	1500.00-4000.00	/mm^3	
Mono %	11.4	2.0-10.0	%	High
Mono (Abs)	500.00	0.00-1000.00	/mm^3	
PNN = Ploymorphonuclear neutrophils, Lymp = Lymphocytes, Mono = Monocytes				

Table 2: Control FBC performed at the CHR Man following the blood transfusion.

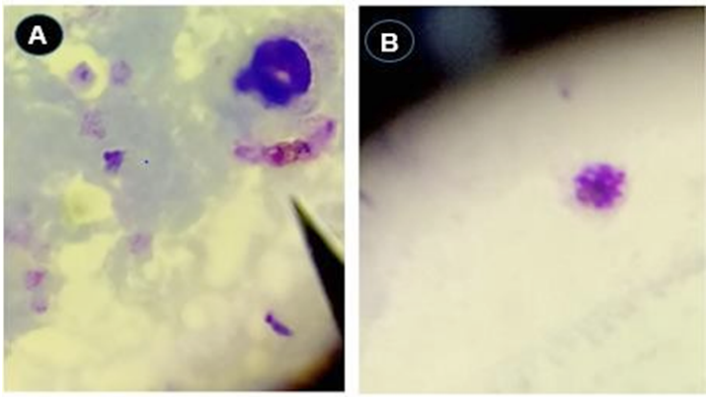

Figure 3: Microscopy of the peripheral blood smear.

A = Gametocyte of *P. falciparum*; B = Schizont of *P. malariae*

The Giemsa stained - thick and thin blood smear on one slide preparation was prepared and read by an experienced laboratory of the CHR Man. This first reader identified a mix slide of 540 Trophozoites of *P. malariae* and *P. falciparum*.

The anaemia was further characterized on the basis the mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and the mean corpuscular volume(MCV) as a severe hypochromic microcytic anemia (Hb < 7g/dl) (NHLBI & NIH, 2022).

Ms. Z.P was released two days later after completing her malarial and transfusion treatmentwith an ambulatory anti anemic treatment (ferric hydroxide polymatose complex syrups).

1 and 2 weeks later Ms. Z.P was visited by the medical team at her home for a follow-up visit; with further follow-up calls every 2 weeks and instructed to visit the nearest health facility should there be any need. She was feeling much better and very playful. As per her mother, since their released from the regional hospital her child condition has tremendously improved.

Over the following month the malaria slide, was sent to the National Institute for Public Health in the capital city (Abidjan) and read by a second experienced technician. He found 741 trophozoites of *P. malariae* and *P. falciparum* (a co-infection) confirming the first reading.

Discussion

Severe malarial anemia caused by *P falciparum* is responsible for approximately a third of the deaths associated with the disease [5] and has been reported in many settings worldwide. Furthermore, it is also well understood that, in higher malaria transmission settings like Côte d`Ivoire, malaria increases the risk of anaemia in the entire population which in turn is a major cause of morbidity and mortality with the greatest impact in young children, and in particular infants [6].

In Côte d`Ivoire, several cases of mixed malarial species infection (*P falciparum*, *P malariae* and O*vale*) have previously been reported *viz.* [7,9]. Recently, several cases of mixed infection *of P. falciparum* and *P. malariae* specires were also reported by Ehouman et al., [10] in the Western region of the country.

In this case report, all 3 RDTs tests used during the screening process and before targeted the HRP 2 (*P. falciparum*) and or pLDHP (*Plasmodium sp*.) rich protein to detect different *Plasmodium* species. And so, the fact all these screening tests failed to detect any plasmodium species may indicated a possibility of a mutation or deletion in the target gene. Similar discrepantfindings (RDT negative and Microscopy positive) have been reported elsewhere. Indeed, Aregawi et al., [11] in the USA reported a diagnosis of *P. Ovale* in a condition where the RDT test wasnegative and the diagnosis of malaria was made on the peripheral blood smear. Berzosa et al., in Equatorial Guinea and Gitonga et al., in Kenya reported similar discrepant results making use of the peripheral blood smear and molecular technic (PCR) to establish their diagnosis [12, 13].

Ms. Z.P beside her severe anaemia (Hg = 4.7 g/dl) did not clinically decompensate. This may be attributed to the fact that at the time of her enrollment in the study she was still on complementary feeding (breast and family foods) and beneficiated from some maternal micronutrients and antibodies that assisted her immunological to remain virtually strong let asideher nutritional deficiencies.

The low parasitemia observed (540-741 trophozoites/ul of blood) may probably due to her immature immune system (3 months to 2 years) relying mainly on the mother`s transmitted immunity protection (maternal antibodies) or the presence of foetal haemoglobin (HbF) which is protective and causes poor parasite growth as previously reported [14,15]. In addition, she had a severe microcytic hypochromic anaemia, which is most commonly caused by iron deficiency and classically appears when iron demand by the bodyis not met by iron absorption from the diet [16]. Furthermore, at 16 months old, Ms. Z.P is still breastfeeding (mixed feed). As per Dalili and co-workers study, infants who are exclusively breast-fed for more than 6 months may be at increased risk of anemia Dalili et al., [17] while Maguire et al., study revealed that the odds of iron deficiency increased by 4.8% (95%IC: 2%–8%) for each additional month of breastfeeding [18]. These may partially explained why her hemoglobin rate is so low.

Conclusion

This case of malaria coupled with a severe hypochromic microcytic anemia (iron deficiency anemia) portrays three main objectives (training, proper diagnosis and appropriate treatment in

poor resourced settings). First, how essential it is for the laboratory technicians to be trained by their institution and to maintain competency in preparing and reading Giemsa-stained thick and thin blood smear at any given time. Secondly, as a reminder of the high prevalence of undiagnosed malarial participants who presented every day at various health centers in various low-income settings globally seeking for assistance. And lastly, the importance of a comprehensive screening to be undertaken by health practitioners in the tropics and subtropics in order to providethe appropriate care (treatment) to those people in need who are is most cases amongst the world'spoorest people.

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