Case Report: Clinical Profile of Concurrent Dengue Fever and *Plasmodium vivax* Malaria in the Brazilian Amazon: Case Series of 11 Hospitalized Patients

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Abstract. Malaria and dengue fever are the most prevalent vector-borne diseases worldwide. This study aims to describe the clinical profile of patients with molecular diagnosis of concurrent malaria and dengue fever in a tropical-endemic area. Eleven patients with concurrent dengue virus (DENV) and *Plasmodium vivax* infection are reported. Similar frequencies of DENV-2, DENV-3, and DENV-4 were found, including DENV-3/DENV-4 co-infection. In eight patients, the World Health Organization (WHO) criteria for severe malaria could be fulfilled (jaundice being the most common). Only one patient met severe dengue criteria, but warning signs were present in 10. Syndromic surveillance systems must be ready to identify this condition to avoid misinterpretation of severity attributed to a single disease.

INTRODUCTION

Malaria and dengue fever are the most prevalent vector-borne diseases worldwide and in the Amazon region of Brazil and heavily impact public health policy. Dengue fever has been an emerging problem in Brazil since the early 1980s, and now all four dengue virus (DENV) serotypes circulate.¹ Malaria remains an important public health challenge in the Amazon Basin as well, with a significant predominance of *Plasmodium vivax* (~85%).²

Malaria and dengue fever must be suspected in all febrile patients living in or returning from the tropics. Concomitant infection has become increasingly common caused by the overlap of vectors in endemic areas and increased prevalence of dengue fever described in recent literature.^{3,4} For this reason, development of diagnostic and treatment approaches for co-infected patients are desperately needed.

Clinical presentation of both uncomplicated malaria and dengue fever are similar, which makes understanding each pathogen's contribution to co-infection difficult. For example, *Plasmodium falciparum*/(DENV) co-infections have been well characterized,^{5,6} but individual contribution to pathogenesis in severe presentations remains unclear. The relationship between *P. vivax*, DENV, and severe clinical presentation is even less well understood. The recent confirmation that *P. vivax* can cause severe or even life-threatening disease,^{7,8} a previously underreported phenomenon, highlights the importance of understanding the dynamics of co-infection.

The first cases of malaria and DENV co-infection came from West Africa (*P. falciparum*)⁹ and India (*P. vivax*).¹⁰ Subsequently, cases in Latin America were reported in French Guiana and Brazil. In French Guiana, a retrospective study with 1,740 patients with acute febrile syndrome identified six confirmed concurrent infection cases, with malaria diagnosis made by thick blood smear and DENV infection diagnosed by positive reverse transcription-polymerase chain reaction (RT-PCR) or viral isolation.⁴ In that study, *P. vivax* and

DENV serotype 3 (DENV-3) were responsible for the majority of the co-infections. In Brazil, in the Amazon region, DENV-2 was detected in sera from two patients living in the Eastern Brazilian Amazon that also presented with acute *P. vivax* infection.¹¹

More recently, Abbasi and others³ reported a large co-infection case series, which suggested that prolonged fever with normal to low hematocrit and marked thrombocytopenia indicate dual infection. These data, however, were based on serological diagnosis, which is not the gold standard for the confirmation of acute DENV infection.¹² Non-specific reactivity for DENV in serological assays cannot be ruled out, as well as positive immunoglobulin M (IgM) related to a recent past infection. Only viral isolation, molecular tests, and/or paired serology in such co-infection episodes are reliable. Further study is also important because the findings may only reflect co-infection dynamics for this specific demographic. The impact of dual infection may vary with local host genetics and viral and parasite serotype.

MATERIALS AND METHODS

This case series aims to describe the clinical course of 11 patients collected over 1 year with molecular diagnoses or NS1 detection of concurrent *P. vivax* malaria and dengue fever. The study was executed in a tertiary health care facility located in the Western Brazilian Amazon, where the four DENV serotypes circulate simultaneously and *P. vivax* is the most commonly diagnosed malaria species.

Patients with the diagnosis of vivax malaria and clinical complications are routinely hospitalized in a ward at *Fundação de Medicina Tropical Doutor Heitor Vieira Dourado* (FMT-HVD), a public tertiary health center for infectious diseases, located in the city of Manaus, the capital of the Amazonas State, in the Western Brazilian Amazon. The institution is a 150-bed reference hospital, including an intensive care unit. From March 2009 to April 2010 (12 months duration), 311 patients with a *P. vivax* diagnosis were hospitalized at the FMT-HVD. Out of these, 132 had available sera stored at –80°C and were also retrospectively tested for DENV.

Enrolled patients of all ages were treated for vivax malaria with chloroquine and primaquine. Severe patients were treated

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with intravenous artesunate and pregnant women received only chloroquine. They were followed daily by the same health team until hospital discharge, and clinical data were recorded in a standard questionnaire for a simultaneous prospective study aimed to characterize clinical presentation of patients with *P. vivax* (data not shown). Patients were classified as severe malaria according to the World Health Organization (WHO) criteria, originally described for severe *P. falciparum* malaria,¹³ and/or as severe dengue fever, according to the 2009 recommendations of WHO.¹² Dengue fever was classified as severe dengue (severe plasma leakage or severe hemorrhage or severe organ impairment) or non-severe dengue, with or without warning signs. Warning signs were defined as abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, or restlessness, liver enlargement > 2 cm, or increase in hematocrit concurrently with rapid decrease in platelet count.

On admission, complete blood count, blood biochemistry, abdominal ultrasound, and chest x-rays were obtained for all patients. Other tests were requested accordingly, e.g., arterial blood gas analysis in the case of respiratory distress. To exclude patients with infection other than *Plasmodium* and DENV, all patients also had a blood culture performed for aerobic bacteria and serological tests for leptospirosis (IgM), HIV-1/HIV-2, hepatitis A (anti-HAV IgM), hepatitis B (HBsAg), hepatitis C (anti-HCV), and hepatitis D (total anti-HDV).

Malaria diagnosis was initially based on a positive thick blood smear on admission. Peripheral parasitemia was quantified as parasites/mm³, using the number of asexual parasites counted in high magnification fields per 500 leukocytes, or the number of leukocytes in 500 counted parasites (depending on what happened first), and the number of leukocytes/mm³ per patient. Afterward, real-time PCR was performed to confirm *P. vivax* mono-infection. The extraction of total DNA from whole blood was performed using the QIAamp DNA Blood

Mini Kit (Qiagen), according to the manufacturer's protocol. The DNA was amplified in an Applied Biosystems 7500 Fast System (Applied Biosystems, Foster City, CA) using primers and TaqMan fluorescence labeled probes for real-time PCR.¹⁴

The DENV diagnosis was based on NS1 detection (enzyme-linked immunosorbent assay [ELISA]) or RT-PCR, when serotype identification was made. The NS1 assays ideally diagnose disease between Days 1 and 6 of symptoms and RT-PCR ideally diagnose disease between Days 1 and 5.¹² Both techniques were used to improve the sensitivity of the specific diagnosis of dengue. Viral RNA was extracted from sera samples using the QIAamp RNA Blood Mini Kit (Qiagen, Germantown, MD), following manufacturer's instructions and stored in -80°C. Reverse transcription, amplification followed by a semi-nested multiplex serotype identification were performed as described elsewhere.¹⁵ When samples were positive for more than one serotype of DENV, a second semi-nested PCR reaction was conducted, in a singleplex format, with only the conserved primer D1 and the type-specific primer (DENV-1 to DENV-4) for confirmation. Amplicons from the C/PrM region were gel purified and sequenced in both directions by using the BigDye Terminator Cycle Sequence Kit (Applied Biosystems). Serotype identification was confirmed with a megablast search to the entire non-redundant nucleotide collection available at GenBank, EMBL, DDBJ, and PDB databases.

CASE REPORTS

During the study period, a total of 11 patients with concurrent DENV and *P. vivax* infection were identified from 132 available tested sera samples (~8.3% positivity). The mean age was 42.7 years (median of 38 years of age) and eight of 11 patients were female, two of which were confirmed to be pregnant. Clinical and laboratory characterization of the patients are described in Tables 1 and 2. All the

TABLE 1

Clinical description of 11 patients admitted to a tertiary health center with confirmed concurrent vivax malaria and dengue fever (Manaus, Brazil, 2009–2010)

Patient	Age/Sex	WHO severe malaria criterion	Warning signs for severe dengue	Duration of fever (days)	Pre-existing medical conditions	Dengue diagnosis	Pregnancy
1	36 y/F	Jaundice, acute lung edema and shock	Dyspnea and abdominal pain	8	–	NS1 +	Yes (Second trimester)
2	40 y/F	Jaundice	Mucosal bleeding, persistent vomiting, and abdominal pain	7	–	DENV-4	No
3	20 y/F	Severe anemia and jaundice	Mucosal bleeding, persistent vomiting, and abdominal pain	4	–	DENV-3	Yes (Second trimester)
4	32 y/M	Jaundice	Mucosal bleeding, persistent vomiting, and abdominal pain	8	–	DENV-3/DENV-4	No
5	23 y/M	Jaundice	Persistent vomiting and abdominal pain	10	–	DENV-3/DENV-4	No
6	16 y/F	–	Mucosal bleeding, persistent vomiting, and abdominal pain	10	–	NS1 +	No
7	63 y/F	Jaundice	Mucosal bleeding, persistent vomiting, and abdominal pain	8	Diabetes, hypertension, and hypothyroidism	NS1 +	No
8	38 y/F	Jaundice	Persistent vomiting	7	–	DENV-3	No
9	46 y/F	–	Mucosal bleeding and persistent vomiting	3	–	DENV-3	No
10	97 y/F	–	–	4	Congestive heart failure	DENV-4	No
11	59 y/M	Jaundice	Abdominal pain	7	Diabetes and hypertension	DENV-2	No

Jaundice: Total bilirubin > 3 mg/dL; Severe anemia: Hemoglobin < 7 mg/dL in adults and < 5 mg/dL in children; NS1: non-structural protein 1 of dengue viruses; DENV-2: dengue virus serotype 2; DENV-3: dengue virus serotype 3; DENV-4: dengue virus serotype 4.

TABLE 2

Laboratory findings of 11 patients admitted to a tertiary health center with confirmed concurrent vivax malaria and dengue fever (Manaus, Brazil, 2009–2010)

Patient	Asexual parasites/mm ³	Hemoglobin (g/dL)	Hematocrit (%)	WBC (×10 ³ /mm ³)	Platelets/mm ³	Serum creatinine (mg/dL)	Serum bilirubin total/conjugated (mg/dL)	AST (IU/L)	ALT (IU/L)	Serum albumin (g/dL)	INR
1	5,450	7.8	25.0	4.8	36,000	1.2	8.5/8.0	56	68	2.9	1.53
2	11,893	10.4	36.2	3.8	13,000	1.4	4.6/3.7	160	268	3.0	1.38
3	7,974	3.0	8.2	4.4	84,000	0.5	3.5/2.8	29	13	3.1	1.17
4	13,202	12.3	41.5	8.6	39,000	1.8	3.5/2.3	62	92	3.1	1.52
5	97	8.3	26.4	6.1	38,000	1.3	5.4/3.6	60	29	3.0	1.19
6	5,237	8.2	24.4	4.2	104,000	0.5	3.2/2.9	23	08	3.0	1.45
7	626	13.5	42.8	6.4	29,000	0.8	9.4/6.3	52	74	3.2	1.09
8	3,984	8.6	24.7	4.9	30,000	0.7	17.6/11.4	39	25	3.0	1.20
9	19,853	11.1	33.0	7.0	97,000	0.6	–	–	–	5.1	–
10	694	9.1	28.1	4.2	90,000	0.9	2.1/1.5	21	22	3.7	1.12
11	15,009	11.0	34.2	3.7	45,000	1.2	10.0/7.2	40	42	3.5	0.95

WBC = white blood count; AST = aspartate aminotransferase; ALT = alanine aminotransferase; INR = international normalized ratio for prothrombin activity
Reference values: hemoglobin: 13.0–16.0 g/dL; hematocrit: 40.0–52.0%; WBC: 4.0–10.8 (×10³/mm³); platelets: 130,000–400,000/mm³; serum creatinine: 0.7–1.5 mg/dL; serum total bilirubin: 0–1.3 mg/dL; AST: 0–38 IU/L; ALT: 0–44 IU/L; serum albumin: 3.5–5.0 g/dL; INR: 0.9–1.3.

patients presented with an acute febrile syndrome with concurrent chills, myalgias, arthralgias, headache, and/or anorexia (Table 3). Eight of 11 patients fulfilled at least one of the WHO severity criteria for malaria, most commonly identified criteria being jaundice (which is defined as total bilirubin higher than 3.0 mg/dL) (Figure 1A). Ten patients presented with at least one of the warning signs for severe dengue, the most common warning sign being severe vomiting (10 of 11), followed by severe abdominal pain (8 of 11), and bleeding (6 of 11) (Figure 1C–E). Diffuse rash was found in 2 of 11 patients (Figure 1F). Dengue diagnosis was confirmed in all cases, but in three patients diagnosis was made with only NS1 positivity, therefore without the identification of the DENV serotype. In two cases DENV-3/DENV-4 co-infection was confirmed by nucleotide sequencing.

On admission thick blood smears were positive for *P. vivax* in all 11 patients, and PCR detection confirmed *P. vivax* infection. No fatalities were observed in our series and the two pregnant women were not followed after hospital discharge for the evaluation of pregnancy complications. Obstetric ultrasound was normal in both. No other co-infections

were found among our patients. No patients required platelet transfusion but red blood cells were transfused to two patients with severe anemia (Hb < 7 g/dL).

The most severe patient was a pregnant woman (patient 1), who presented with respiratory distress, pleural effusion, hypotension, and jaundice. Despite having marked thrombocytopenia (36,000 platelets/mm³), she had no spontaneous bleeding. This patient received intravenous crystalloids for fluid resuscitation, which precipitated acute lung edema (Figure 1B) that was subsequently treated with diuretics, which achieved good clinical recovery.

DISCUSSION

It has already been shown that *P. vivax* monoinfection is responsible for severe disease in Manaus,¹⁶ and it is hypothesized that concurrent DENV infection may overestimate the burden of severe vivax disease. This hypothesis is based on the increase in hospitalizations attributed to vivax infection during the mid 1990s, when DENV-1 was first identified in Manaus.^{17,18} Since the 1990s, DENV-2 and DENV-3 were also gradually introduced, and in 2008 DENV-4 was reintroduced in Brazil.¹ All four serotypes now circulate; thus, increasing the likelihood of subsequent outbreaks, severe dengue, and co-infections with other equally prevalent infectious diseases. Malaria and bunyavirus infections have been described since 1971.¹⁹

The RT-PCR positivity for DENV even in patients with more than 7 days of disease may not only be related to the sensitivity of this technique, but also point to the fact that the total number of days of sickness referred by the patient may be over attributed to a single disease. Thus, it is possible that the initial febrile syndrome is caused by one disease and persists longer because of another. The other limitation to the clinical approach to co-infection is the frequent occurrence of asymptomatic carriers of *Plasmodium* sp. in endemic areas,²⁰ raising the possibility that the present clinical picture detected by the clinician was eventually related only to dengue fever. Whether one infection could enhance the risk of acquiring the other or whether they are purely two random events occurring at the same time is still speculation.

Another finding was the identification of multiple DENV serotype infection in 2 of 11 patients. Although DENV hyperendemicity (with the simultaneous circulation of different

TABLE 3

Frequency of signs and symptoms presented by 11 patients admitted to a tertiary health center with confirmed concurrent vivax malaria and dengue fever (Manaus, Brazil, 2009–2010)

Signs and symptoms	n/N (%)
Fever	11/11 (100)
Anorexia	11/11 (100)
Chills	11/11 (100)
Headache	11/11 (100)
Myalgia	10/11 (91)
Arthralgia	10/11 (91)
Vomiting	8/11 (73)
Abdominal pain	8/11 (73)
Jaundice	8/11 (73)
Cough	5/11 (45)
Diarrhea	4/11 (36)
Metrorrhagia	3/11 (27)
Rash	2/11 (18)
Oliguria	2/11 (18)
Dyspnea	1/11 (9)
Petechiae	1/11 (9)
Ecchymosis	1/11 (9)
Gingival bleeding	1/11 (9)
Conjunctival and retinal bleeding	1/11 (9)
Hypotension	1/11 (9)

FIGURE 1. (A) Icterus in the ocular mucosae of patient 12 (total bilirubin 10.0 mg/dL); (B) thorax roentgenogram of patient 1, in the second trimester of pregnancy, evolving with acute lung edema after fluid therapy for the treatment of shock; (C) petechiae in the lower limbs in patient 1; (D) conjunctival bleeding in patient 3, in the second trimester of pregnancy; (E) retinal bleeding in patient 3, in the second trimester of pregnancy and vision blurring; (F) diffuse rash in patient 7, evolving with intense pruritus.

viruses) is quite common in tropical areas, co-infection with multiple viruses has been scarcely reported with a few case reports from Taiwan, Puerto Rico, and Brazil.^{21–23} In 2011, this phenomenon was reported during an outbreak in our region, and was not related to severity.²⁴

Even without the proper design to estimate if co-infection is associated with more severe complications, this case series describes a high frequency of persistent vomiting, abdominal pain, and bleeding, which are well-known warning signs for severe dengue.²⁵ Severity criteria for vivax malaria seem to be the same as those originally used for *P. falciparum*, as shown elsewhere,²⁶ and were also common in this case series.

Respiratory distress is an increasingly reported complication in vivax malaria,²⁷ and acute lung edema and pleural effusion are common clinical features of severe dengue. Clinical management may prove difficult in these cases because of the amount of fluids necessary to treat severe dengue shock without inducing fluid overload, as seen in patient 1.

Cholestatic jaundice, as seen in Figure 1A, was also a very frequent complication observed in our case series (8 of 11), and it is not an unusual accompaniment of vivax malaria, probably caused by hepatocyte dysfunction.^{28,29} Despite being common in both *P. falciparum* and *P. vivax* infection, jaundice as an individual marker of severity is widely questioned,³⁰ unless

accompanied by failure of another organ system.¹³ In contrast, jaundice is rarely seen in severe DENV infection despite viral tropism for hepatocytes.^{31,32} However, when jaundice is present in DENV infection, it may be considered as a sign of severe disease.^{33,34} This dichotomy and the high rates of co-endemicity, indicate that jaundice could serve as a sign of co-infection. Similarly, the presence of rash in malarial patients (as seen in Figure 1F) might suggest any exanthematous viral disease, e.g., dengue fever, parvovirus B19, or rubella.¹⁸

A recent case-control retrospective study in French Guiana pointed to more severe anemia and thrombocytopenia in patients with the co-infection,³⁵ however, a larger series of cases in different settings are needed to validate this finding. Mild spontaneous bleeding, as seen in Figure 1C–E, was present in 6 of 11 patients in this study and only half of these patients had severe thrombocytopenia (also considered as a warning sign). This reinforces our previous finding that the degree of thrombocytopenia does not necessarily correlate with clinically significant bleeding, suggesting that factors such as liver and vascular injury may also contribute to dengue associated bleeding diatheses.³⁶ There is no evidence that thrombocytopenic patients with malaria benefit from platelet transfusions.^{37,38} Similarly, the management of severe

thrombocytopenia in dengue patients (platelet count under 50,000/mm³) also lacks evidence-based treatment guidelines. The recommendation of prescribing platelet transfusion based solely on the presence of hemorrhage sufficient to cause a decrease in hemoglobin level of 3.0 g/dL, a decrease in blood pressure, bleeding into a vital organ, for those who underwent invasive care procedures, or for patients with any bleeding and very low platelet count (under 5,000/mm³), seems to be safe for adult dengue patients.^{36,39,40} In this case series, patients 2 and 7 presented non-life threatening, spontaneous bleeding in the setting of thrombocytopenia, but both responded well to conservative management. In a series of 17 confirmed co-infection cases in French Guiana with an epidemiological profile similar to our Manaus, thrombocytopenia was the major complication observed in retrospective data from emergency room patients.⁴

Severe anemia was observed in 1 of 11 patients and required red blood cell transfusion. The clinical use of hemoglobin/hematocrit as a severity marker in concurrent DENV and malaria infection could be tricky and may cause some misinterpretation, considering that both diseases impact red blood cell count through different mechanisms that should be addressed individually. Therefore, hemoconcentration may not have the same relevance for evaluating potential severe dengue in patients with malarial anemia. Similarly, severe anemia observed in malaria may appear falsely normal because of plasma leakage in severe dengue fever.

The impact of chronic diseases on dengue fever severity has already been addressed in Brazil^{1,41} and these co-morbidities, mainly diabetes, allergy, and hypertension, may play an important role in determining severity for DENV infection. The impact on vivax disease, however, is poorly described and needs further study. Pregnancy is clearly recognized as a risk factor for severe DENV infection leading to obstetric complications,⁴² but scarce literature is available on the impact of *P. vivax* on the course of gestation.⁴³ In our series, 2 of 8 female patients were pregnant and both presented with complicated disease, but no pregnancy outcome was evaluated prospectively. Larger series are required to estimate if this subpopulation is more susceptible to severe disease during the co-infection.

The absence of fatalities and/or severe bleeding requiring blood transfusion in our study may reflect the facilitated access to malaria diagnosis and prompt specific treatment in the Amazon region, and the early identification and hospitalization of potential severe cases in a public tertiary health care facility.

Dengue and malaria co-infection requires special attention because delayed diagnosis and inappropriate treatment may result in fatal complications.⁴⁴ The diagnosis of the co-infection also points to the low efficacy of local control measures, which could be reinforced to synergize with improved diagnosis and treatment techniques. The increase in co-infection cases also reflects the recent impact of urbanization on vector-borne diseases.⁴⁵ Malaria, a traditionally rural disease in Latin America, is becoming more peri-urban,⁴⁶ and therefore tends to co-exist with a typical urban endemic disease, such as dengue fever.

CONCLUSIONS

This case series shows that concurrent DENV and *P. vivax* infection occurred in ~8% of hospitalized vivax malaria

patients in the Western Brazilian Amazon, suggesting that this may not be a rare phenomenon in tropical endemic areas in Latin America. For this reason, identifying signs of severity is of the utmost importance, and to better guide patient management, knowing which infectious etiology is the primary driver for each sign. Furthermore, the variability of clinical presentation highlights the need for future studies to define the range of manifestations across multiple demographics.

Severity signs could be attributable to co-infection, misclassifying severe vivax malaria, and/or severe dengue fever; however, the next step of investigation should focus on larger multicenter case-control studies with the potential to evaluate if co-infection actually leads to more severe disease.

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