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ORIGINAL ARTICLE

Dengue: etiology of acute febrile illness in Abidjan, Côte d'Ivoire, in 2011–2012

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Background: The burden of dengue in Africa is not well understood. A prospective study was conducted in Abidjan, Côte d'Ivoire from December 2011 to December 2012 to estimate the proportion of dengue and malaria cases among febrile patients during a period when dengue was not known to be circulating in the region, and to describe the clinical and virological characteristics of laboratory-diagnosed dengue cases.

Methods: Blood samples were taken from febrile patients (body temperature ≥38°C) at two study sites. Patients with fever lasting more than 7 days, with fever of known origin and with jaundice were excluded. Thick blood film tests, ELISA for anti-dengue IgM and reverse transcription-PCR (RT-PCR) were performed.

Results: A total of 812 patients were enrolled (51.7% male [48.3% female]; 46.4% aged <10 years) of whom 796 (98.0%) provided IgM ELISA and RT-PCR data, and 807 (99.4%) had thick blood film results. Three (0.4%) patients had laboratory-diagnosed dengue (one with DENV-3 serotype), none of whom were diagnosed clinically, and 234 (28.8%) had confirmed malaria.

Conclusions: This study suggests that dengue virus circulates in Abidjan outside an epidemic and that there should be an increase in awareness of dengue as a possible diagnosis in cases of undifferentiated fever. These results stress the importance of implementing laboratory capacity to assess dengue burden in Africa.

Keywords: Côte d'Ivoire, Dengue, Fever, Malaria, Prevalence study

Introduction

Dengue is a systemic infection caused by a virus belonging to the Flaviviridae family (genus, Flavivirus). Four serotypes of dengue virus (DENV) exist, DENV-1 to DENV-4. Transmission of the virus occurs via bites from the *Aedes* genus of mosquitoes, principally *Aedes aegypti*, a cosmotropical species that inhabits urban areas in proximity to housing.[1]

Dengue is currently the most widespread arbovirosis in the world, and is a major public health problem in tropical and subtropical regions. Dengue infections are frequently asymptomatic and patients who present with symptoms can be misdiagnosed; hence, there is under-reporting of the true number of infections. One recent report estimated that in 2010, 390 million dengue infections occurred, of which 96 million were symptomatic cases.[2] An evidence-based consensus study estimated that dengue virus transmission occurs in 128 countries worldwide, with the population potentially at risk of dengue infection being 3.97 billion.[3]

Dengue virus circulation has been reported in 34 countries in Africa, and dengue is common in travellers returning from Africa.[4] In Côte d'Ivoire, there was a reported case of dengue virus isolation from the blood of a young soldier living in Abidjan,[5] as well as serological evidence of previous dengue infection (11 cases positive for anti-dengue IgG and one positive for IgM) in blood samples from mosquito catchers living in the Comoe national park area in the North Eastern part of the country.[6] Furthermore, a study of probable and confirmed imported cases of dengue between 1994 and 1999 in France showed that nine out of 17 cases imported from Africa were in travellers returning from Côte d'Ivoire.[7] Subsequently, between 1 July 2006 and 31 December 2008, an increase in the number of dengue cases imported into France from the Côte d'Ivoire, was reported.[8] In

2008, two imported cases in travellers returning from Côte d'Ivoire were recorded in Japan and France.[8] Serological and reverse transcription-PCR (RT-PCR) tests of presumed dengue cases returning from or living in Abidjan, in whom anti-yellow fever IgM was undetectable, helped reveal the circulation of the DENV-3 serotype in Côte d'Ivoire in 2008.[9,10] These cases occurred during a yellow fever epidemic and, following this re-emergence of the yellow fever virus, entomological investigations showed that the *Aedes aegypti* vector accounted for a high proportion of the mosquito population in Abidjan.[11] Following the 2008 epidemic, it became mandatory to test for dengue as a differential diagnosis for yellow fever in Côte d'Ivoire, and dengue is now in the process of being added to the list of reportable diseases at the national level. More recently, a retrospective study identified three IgM-positive and four PCR-positive dengue cases in Abidjan in 2010.[12]

To our knowledge, no data are available on the incidence or prevalence of dengue disease in Côte d'Ivoire. It therefore appeared timely to carry out research on the dengue virus, in order to make reliable and up-to-date data available to the local health authorities, which will allow them to make appropriate decisions to protect the population. This article describes a study carried out in Abidjan, Côte d'Ivoire in 2011–2012 to assess the proportion of dengue and malaria cases among febrile patients during a period when dengue was not known to be circulating in the region, and to describe the clinical characteristics and infecting serotypes in laboratory-diagnosed dengue cases.

Materials and methods

Study design

This was a descriptive, prospective, two-centre study carried out in Abidjan, the economic capital of Côte d'Ivoire, situated on the south coast of the country. The city of Abidjan is separated into northern and southern areas by the Ebrié lagoon, and one study site was chosen from each area. The first was Hopital Général de Koumassi, a public hospital in the Koumassi district of southern Abidjan, which is close to the airport and borders the Ebrié lagoon. Patients were recruited from outpatient clinics in general medicine and paediatrics. The second site was Polyclinique Internationale Sainte Anne Marie (PISAM), a private clinic situated in the Cocody district in northern Abidjan, a more affluent area of the city, located in the hills. Patients at PISAM are typically wealthier than average, and the clinic also treats patients from neighbouring countries. Both inpatients and outpatients were recruited at PISAM, the latter from general medicine and paediatrics clinics. The enrolment period lasted 12 months, from 12 December 2011 to 14 December 2012. The study was approved by the national ethics committee (Comité national d'éthique ivoirien) and conducted according to the principles of the Declaration of Helsinki (Edinburgh amendment, October 2000).

Study population and inclusion and exclusion criteria

For practical purposes and to minimize disruption to routine practice at the two sites, the target enrolment was set at 800 febrile patients over a 12-month period: 600 from the public hospital and 200 from the private clinic. This was estimated to represent about 2% of all acute fever cases seen at these two sites annually (based on the number of admissions with fever at the sites in previous years). To reach this number, patients were recruited during one week in each calendar month. The inclusion calendar was set before the start of the study, and the inclusion weeks were randomly chosen. During recruiting weeks, all patients presenting with recent acute fever were invited to participate.

Patients of any age who presented with fever (body temperature $\geq$38°C) of up to 7 days' duration or reported fever in the past 7 days, with or without haemorrhagic symptoms, were eligible for inclusion. Patients with fever of known bacterial or parasitic origin and patients with jaundice were excluded. All study participants (or the parent or legal guardian of children <18 years old) gave written informed consent to participate; a witness connected to the family could give consent on behalf of illiterate patients or parents.

Data collection

On the day of study entry, patients underwent a clinical examination and the following data were collected: visit date, body temperature recorded during visit, start date of fever, clinical signs, and the clinical diagnosis established at the end of the visit. Two venous blood samples were collected from each patient (5 mL in patients older than 10 years and 3 mL for those under 10 years): the first was used for haematological and biochemical tests and screened for the presence of Plasmodium parasites by microscopic examination using the thick blood film technique. These tests were carried out locally by the laboratory at each study site according to its own procedures. A confirmed malaria case was defined as a patient with a positive thick blood film result (i.e., presence of the malaria parasite). The second sample was sent to Institut Pasteur de Côte d'Ivoire, which performed a serological screen for anti-dengue virus IgM using Centers for Disease Control dengue IgM antibody capture-ELISA (MAC-ELISA),[13,14] as well as a genome screen for dengue virus types 1–4 by fluorogenic RT-PCR using the technique described by Ito et al.[15] If at least one of these two screens was positive, the patient was classed as a laboratory-diagnosed dengue case, and viral isolation was carried out by means of culture in Vero E6 and PS (porcine stable) cell lines.[16]

Descriptive statistical analyses were carried out with Stata Statistical Software, Release 12 (StataCorp LP, College Station, TX, USA).

Results

Study population

A total of 812 patients were included in the study from December 2011 to December 2012. Of these, 609 patients were enrolled at the Koumassi site and 203 at PISAM. All patients at Koumassi were ambulatory and all patients at PISAM were hospitalized. The study sample consisted of 392 (48.3%) female patients and 420 (51.7%) male patients. The most common age group represented was children <10 years old (377/812; 46.4%); distribution of all patients by age group is shown in Table 1. Of the 812 study patients, 488 (60.1%) lived in the Koumassi district.

The mean fever duration from onset to hospital visit was 2.1 days and 792 (97.6%) patients presented at the hospital within 5 days of the start of fever. At the time of clinical examination, 731 (90.0%) patients had a body temperature of $\geq$38°C. The

Table 1. Patient characteristics at study entry (n=812)

	Number (%) of patients
Male patients	420 (51.7)
Age range, years	
0–9	377 (46.4)
10–19	78 (9.6)
20–29	103 (12.7)
30–39	104 (12.8)
40–49	78 (9.6)
50–59	43 (5.3)
60–69	25 (3.1)
>69	4 (0.5)
Presenting symptoms	
Headache	508 (62.6)
Myalgia	454 (55.9)
Arthralgia	300 (36.9)
Vomiting	260 (32.0)
Abdominal pain	213 (26.2)
Cough	208 (25.6)
Cold symptoms	109 (13.4)
Retro-orbital pain	98 (12.1)
Diarrhea	73 (9.0)
Shivering	31 (3.8)
Rash	26 (3.2)
Haemorrhagic manifestations[a]	13 (1.6)
Pleural effusion	1 (0.1)
Platelet counts	
<50 000/mm^3	21 (2.6)
50 000–99 999/mm^3	63 (7.8)
100 000–149 999/mm^3	135 (16.8)
≥150 000/mm^3	584 (72.7)

[a] Including epistaxis (6[0.7]), bleeding gums (1[0.1]), hematemesis (3[0.4]), melena (3[0.4]), other bleeding (4[0.5]).

most common symptoms at presentation were headache (reported by 62.6% [508] patients) and myalgia (454, 55.9%); the numbers reporting other symptoms are shown in Table 1. Thirteen (1.6%) patients had haemorrhagic manifestations and one (0.1%) patient had non-bloody pleural effusion.

In 219 (27.3%) of 803 patients tested, platelet counts were below normal (<150 000/mm^3) (Table 1). Elevated haematocrit (>54.0% for male patients and >47.0% for female patients) was recorded in nine (1.5%) of 600 patients tested. Concentrations of aspartate and alanine aminotransferases (ASAT and ALAT; upper limit of normal range, 40 IU/L) were measured in 200 hospitalized patients: of these, 81 patients had elevated levels of ASAT (highest reading, 812 IU/L) and 77 had elevated levels of ALAT (highest reading, 920 IU/L).

Based only on clinical observation (before laboratory results were available), 409/812 (50.4%) patients were clinically diagnosed with malaria, 130 (16.0%) with anaemia and 119 (14.7%) with respiratory disease. A variety of other clinical diagnoses were made for the other 154 patients, with no single diagnosis accounting for more than 10 cases. No patient was diagnosed as having dengue based on clinical observations.

Table 2. Malaria test results grouped by clinical and laboratory diagnoses (n=812)

	Malaria test result (thick blood film)			
Clinical diagnosis	Positive	Negative	Not tested	Total
Malaria	160	246	3	409
Other or no diagnosis	74	327	2	403
Total	234	573	5	812

Dengue and malaria laboratory test results

Results of the anti-dengue virus IgM assay and RT-PCR were available for 796 (98.0%) of 812 patients. Two patients were positive for anti-dengue IgM and one patient was positive for RT-PCR, giving a total of three laboratory-diagnosed dengue cases (0.4%).

Thick blood film tests were done on samples from 807 (99.4%) patients, of which 234 (29.0%) were positive. Of the 234 confirmed malaria cases, 160 (68.4%) were clinically diagnosed as suspected malaria cases whereas 74 (31.6%) were not. Also, among the 406 clinically suspected cases of malaria who were laboratory-tested, 160 (39.4%) had a positive thick blood film (Table 2).

Description of laboratory-diagnosed dengue cases

The three laboratory-diagnosed dengue cases are described here in more detail.

Case 1

The first case, a 53 year-old man from the Cocody district, was identified on 8 August 2012 from a dengue positive IgM blood sample; his RT-PCR was negative. He was admitted to PISAM hospital on the third day of fever onset. He presented with fever (38.8°C), headache, myalgia, arthralgia, retro-orbital pain, vomiting and abdominal pain. His platelet count was below normal (129 000/mm^3), a haematocrit of 40%, and elevated liver enzymes (ALAT 60 IU/L; ASAT 56 IU/L). His clinical diagnosis was malaria and urinary infection, but his thick blood film test was negative. After four days in hospital, he was discharged, with a diagnosis of viral disease and urinary infection. Viral isolation was negative.

Case 2

The second case, a 5 year-old boy from the Koumassi district, was identified on 25 September 2012 from a positive IgM blood sample; his RT-PCR was negative. He was seen in an ambulatory visit at the Koumassi Public Hospital on the second day of fever onset. He presented with fever (38.5°C), headache, myalgia and vomiting. He had a normal platelet count (211 000/mm^3) and a haematocrit of 37%. No liver enzyme tests were done. His clinical diagnosis was malaria, but his thick blood film was negative. Viral isolation was negative.

Case 3

The third case, a 39 year-old man who worked on an offshore oil platform, was identified on 28 October 2012 from a positive

RT-PCR blood sample; his IgM test was negative. He was admitted to PISAM hospital on the second day of fever onset. He presented with fever (39.7°C), headache, myalgia and arthralgia. His haematologic test results at admission were in normal ranges: platelet count, 167 000/mm^3; haematocrit 42%; ALAT 24 IU/L; and ASAT 21 IU/L. His thick blood film was negative. DENV-3 serotype was identified by RT-PCR, but viral isolation was negative. After five days in hospital, he was discharged; the clinical and discharge diagnose had been suspected salmonella infection.

All the laboratory-diagnosed dengue cases recovered and were discharged.

Discussion

Between December 2011 and December 2012, three dengue cases were identified among 812 febrile patients in Abidjan. The three laboratory-diagnosed dengue cases presented with fever of ≥38°C during clinical examination, and with headache and myalgia. Two of the cases also presented with arthralgia, retro-orbital pain and abdominal symptoms. The patient with a positive RT-PCR result was infected with the DENV-3 serotype, but virus culture results were inconclusive in all three laboratory-diagnosed cases. The proportion of laboratory-diagnosed dengue cases in our study was therefore 0.4%, and none of the patients were clinically diagnosed with dengue fever. IgM-based assays have been shown to have about 90% sensitivity and 98% specificity when undertaken five days or more after the onset of fever in areas, such as the Côte d'Ivoire, where dengue is not endemic as any positive result likely indicate recent infections.[17] However the possibility that the IgM reactivity was non-specific or cross-reactive (i.e., due to infection with another flavivirus) cannot be excluded. Despite the small number of laboratory-diagnosed cases, these results suggest that dengue virus is circulating in Abidjan. Malaria remained the main cause of febrile illness in our study. Laboratory results showed that 29% of patients had malaria, whereas the proportion that had been clinically diagnosed with malaria was 50.4%.

Several factors favour transmission of dengue virus in Côte d'Ivoire and more generally in Africa. The mosquito vector is widespread,[4] and demographic and socioeconomic changes in recent decades, such as population growth and urbanization, create favourable conditions for increases in the population of the mosquito vector. Dengue was first reported in Africa at the end of the 19th century and viral circulation was retrospectively confirmed for the first time in the mid-1950s after detection of anti-dengue virus antibodies in samples taken during an epidemic in South Africa in 1926–1927.[4] Current data suggest that dengue circulates in 34 countries in Africa.[4] However, to date, the only reported large-scale epidemic occurred in 2009 in Cape Verde (17 000 cases; 6 deaths) caused by the DENV-3 serotype.[18] Subsequent phylogenetic analysis of DENV-3 serotypes circulating in West Africa showed that the viral strain responsible for the 2008 outbreak in Côte d'Ivoire was closely related to the virus that caused the Cape Verde epidemic,[18] suggesting that this serotype was circulating in the region in 2008–2009.[8,10]

Although conditions are favourable for dengue virus circulation and transmission in Africa, the number of dengue cases reported to WHO remains lower in Africa than in South-East Asia or Latin America.[1] Quantifying the seroprevalence based on serological tests may be complicated by cross-reactivity to other flaviviruses.[19] Consequently, the prevalence of anti-dengue IgGs can be overestimated by false positive results due to circulating yellow fever and West Nile viruses, and by natural antibody responses after anti-yellow-fever vaccination campaigns.[20] This may be partly responsible for the wide variation in results of recent seroprevalence studies in African countries other than Côte d'Ivoire, although differences in the age and health status of the population studied are also contributors. For example, a study of 4341 people in 220 villages in Gabon between July 2005 and May 2008 reported a prevalence of 0.5% for both anti-dengue IgG and IgM, even though a DENV-2 epidemic occurred in the country in 2007, combined with a chikungunya epidemic.[21] Studies in Kenya have reported estimates of anti-dengue IgG seroprevalence of 14.4% in adults, from a 2004 study in 1141 individuals,[22] and 1.1% in healthy children aged 12–47 months,[23] but interpretation of these results is uncertain given the potential for cross-reactivity with other flaviviruses. In a seroprevalence study conducted in Cameroon in 2007, one year after reports of dengue-like illness in the north-west of the country, no anti-dengue IgG or IgM was detected in any samples from 105 adults.[24] An earlier study in Cameroon, conducted between 2000 and 2003 in 256 adults living in rural villages reported 12.5% seroprevalence based on a plaque-reduction neutralization test.[25] Thus, in spite of the limitations of assay cross-reactivity, the data suggest that dengue virus circulates in Africa at low levels.

A retrospective study was performed to detect anti-dengue IgG and IgM in 95 serum samples from patients in Bamako, Mali, who presented with a febrile episode of unknown origin, and for whom malaria was excluded on the basis of laboratory tests. Among these samples, 93% were anti-dengue IgG positive and none were positive for anti-dengue IgM.[26] In addition, dengue nonstructural protein 1 (NS1) antigen was detected in one case in that study.

Several hypotheses have been put forward to explain the low number of dengue cases and the low levels of seroprevalence in Africa. It is possible that lack of appropriate laboratory testing for dengue may contribute to the low numbers reported. In Côte d'Ivoire, malaria is endemic, and transmission occurs throughout the country at a high or very high level; WHO estimated the annual number of malaria cases in 2010 to be nearly 7 million.[27] Therefore, there is a risk of overdiagnosis of malaria by medical personnel, which may lead to under-recognition and underdiagnosis of other acute febrile illnesses including dengue.[4,28,29] Dengue appears to be less well-known to the local population, and more than 70% of fevers are treated at home with traditional remedies and without recourse to the health system.[4,30] Another possible reason is that genetic factors may play a role in the clinical expression of dengue infection, given that people with African ancestry are reported to be less susceptible to severe forms of dengue.[31]

Clinical diagnosis of dengue is difficult in areas where other febrile illnesses are endemic, as the clinical signs are not specific.[29] Dengue symptoms that are frequently observed in the febrile phase, like arthralgia, myalgia or headaches, are observed in other acute febrile illnesses in Africa such as malaria, yellow fever and influenza. It is therefore important for caregivers to have an effective laboratory test for diagnosing dengue, particularly in regions where several pathogens circulate. For the moment, the availability of rapid serological diagnostic tests for dengue which are quick and require minimal user expertise should be considered. RT-PCR and viral culture provide laboratory

confirmation with good specificity and sensitivity for a sample that is taken in the early stages of fever, although they are costly and difficult to implement in tropical and subtropical countries, particularly during an epidemic.

As our study was conducted in only two hospital sites in Abidjan over one year, we cannot be confident that these findings can be generalized to the whole of Côte d'Ivoire. The travel history of patients was not available, which could have limited the value of some of our results. Additionally, the large majority of samples were taken between one and five days after of the start of fever, whereas IgM antibodies are not usually detectable until five days after fever has started. This could potentially lead to an underestimation of the number of dengue cases among these febrile patients. As such, both acute and convalescent phase specimens may be needed to confirm dengue infection and the absence of convalescent samples was a limitation of our study. Furthermore, interpretation of the results of serological antibody assays may be confounded by cross-reactions.[19] Collection of blood samples on filter paper and diagnosis with NS1 antigen assays may help overcome some of these limitations.[32] In addition, RT-PCR testing requires strict storage and transportation conditions that are not easy to ensure in African countries such as Côte d'Ivoire. To minimize these problems, our samples were transferred daily to Institut Pasteur de Côte d'Ivoire laboratory with temperature monitoring.

Conclusions

This study suggests that dengue virus circulates in Abidjan, Côte d'Ivoire, outside of an epidemic. Despite the low proportion of dengue cases identified among febrile patients, these results add to the knowledge base of dengue prevalence in Africa. Moreover, dengue was not diagnosed clinically in any of the three laboratory-diagnosed cases, which stresses the importance of raising the awareness of clinicians and improving the availability of sensitive, specific and easy-to-use diagnostic tests to reliably assess the burden of dengue infection in Africa.

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Ethical approval: The study was approved by the national ethics committee (Comité national d'éthique ivoirien) on 28th October 2009 and a subsequent amendment was approved on 29th December 2011.

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