Contribution of Dengue Fever to the Burden of Acute Febrile Illnesses in Papua New Guinea: An Age-Specific Prospective Study

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Abstract. Malaria is a major contributor to the burden of febrile illnesses in Papua New Guinea (PNG). Dengue fever (DF) is likely to contribute; however, its epidemiology in PNG is poorly understood. We performed a prospective age-stratified study in outpatient clinics investigating the prevalence of DF; 578 patients were enrolled, and 317 patients with a negative rapid diagnostic test (RDT) for malaria were tested for dengue. Malaria was confirmed in 52% (301/578, 95% confidence interval [CI] = 48–56%), DF was diagnosed in 8% (46/578, 95% CI = 6–10%), and 40% (95% CI = 36–44%) had neither diagnosis. Among the 317 malaria RDT-negative patients, 14% (45/317, 95% CI = 10–18%) had DF. The seroprevalence of dengue immunoglobulin G (IgG) was 83% (204/247, 95% CI = 78–87%), and no dengue hemorrhagic fever was seen. This study provides good evidence for the first time that DF is common in PNG and is responsible for 8% of fever episodes. The common occurrence of DF in a population with presumed previous exposure to dengue is an important observation.

INTRODUCTION

After the Second World War, urbanization and increased population densities facilitated the circulation of dengue virus. Dengue fever (DF) has become a growing problem, especially in Asia and America, with millions of cases every year and the emergence of outbreaks of dengue hemorrhagic fever (DHF).¹

Dengue virus belongs to the flavivirus genus (family flaviviridae) and is closely related to other flaviviruses such as Japanese Encephalitis, tick-borne encephalitis, and West Nile virus.

The classification of dengue illnesses is being revised; however, three different forms of the disease have traditionally been described: DF, DHF, and dengue shock syndrome (DSS).² They are not independent diseases but represent a continuum from a mild undifferentiated febrile illness to a severe disease with microvascular plasma leakage leading sometimes to death. The criteria have been criticized because of their rigidity and difficulties in their application in clinical practice.^{3,4}

Classic DF has an incubation period of 2–7 days followed by the sudden onset of high fever, retro-orbital headache, macular rash along with arthralgia and myalgia, skin hyperesthesia, change of taste perception, and anorexia. At the end of the illness, at the time of fever lysis, there may be an exacerbation of rash and desquamation of the skin on the hands. A long-lasting asthenia may also follow the end of the febrile disease. DF can be associated with epistaxis or petechiae, which is a consequence of thrombocytopaenia. It is recognized that asymptomatic infections are thought to be uncommon, at least in adult Caucasian and Asian populations.^{5,6} However, several studies have shown that the rate of asymptomatic infections can be much higher in the pediatric population.⁷

Since the 1960s, millions of people have been affected by DF, and many have died as a consequence of DHF. Different regions of the world are differently affected, and outbreaks of

DF usually precede the emergence of DHF.⁸ The epidemiological relationship between DF and DHF is complex and not fully understood. Several hypotheses have been formulated. The most favored is called the two-infections phenomenon, and it is supported by evidence that the risk of developing a DHF is higher in patients exposed in the past to different serotypes of dengue.^{9,10} This is believed to be the consequence of a non-neutralizing antibody-dependant infection enhancement.¹¹ Other factors have been identified to be associated with DHF, such as the virulence of the dengue virus strain and host genetic factors like human leucocyte antigen (HLA) type.¹²

DF has been widely reported and documented in many of Papua New Guinea's (PNG) neighboring countries, and it is a concern among the health authorities of those nations. In these Pacific Island nations, DF normally occurs in epidemics. In 2009, Fiji, French Polynesia, and New Caledonia were affected.¹³ DF is endemic in some countries (e.g., Indonesia, the Philippines, and New Caledonia).^{14–17} PNG is similar in that there are large populations of the vectors *Aedes aegypti* and *Ae. albopictus*¹⁶ and that there are favorable environmental conditions for the mosquito to proliferate and interact with humans, such as warm climate, poor sanitation, and overcrowding. A dengue type 2 outbreak was first reported in the country in 1971–1972,^{18,19} another dengue outbreak was reported in 1976, and the last reported dengue outbreak occurred in 1983.²⁰ Occasional cases of dengue acquired in PNG have also been reported by health authorities in Cairns, Australia.²¹ Although there is evidence that DF is present in PNG, no information is available with respect to the pattern of the disease and its importance in the clinical practice. Importantly, DHF has never been reported in the country, despite its occurrence in the neighboring region of Irian Jaya (Indonesia).²² The diagnosis of dengue is not usually considered by clinicians, and malaria is routinely the only diagnosis mentioned to explain febrile illnesses.

In this study, we conducted a prospective age-stratified study in two urban and rural outpatient clinics in Madang Province (PNG) with the aim of investigating the contribution of DF to the burden of febrile illness in patients who did not have malaria.

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SUBJECTS, MATERIALS, AND METHODS

Study population. We conducted a prospective clinical study in the Madang province, PNG, from October 2007 to June 2008. The two sites were the outpatient clinic of Yagaum rural hospital, located about 20 km from Madang town and mainly serving a rural population, and the Jomba town clinic in Madang town, serving poor people from surrounding urban settlements. The protocol was approved by the Institutional Review Board of the Institute of Medical Research (8/28/2007, IMRIRB 0715) and the Medical Research Advisory Committee of the National Department of Health (MRAC, number 07.21, 8/28/2007).

Participants were eligible for recruitment from the outpatient clinics at both sites when presenting with fever, which was defined as an axillary temperature $> 38^{\circ}\text{C}$ or a history of fever in the past 5 days. Participants were categorized in specific age groups: < 1 , 1–4, 5–9, 10–14, 15–24, and > 25 years. Written informed consent was obtained from all participants (or from the parents of children < 16 years) before any data collection. Study participants were consecutively recruited only during weekdays when a study nurse was present (Monday to Friday).

Data and sample collection. Demographic data (name, age, gender, and location) and relevant clinical signs and symptoms of the current illness were collected using an in-depth medical questionnaire administered by a trained study nurse. All participants also underwent a brief clinical examination by the nurse, and all relevant signs were recorded (signs of bleeding, rash, lymphadenopathy, rhinitis, cough, respiratory rate, presence of jaundice, and abdominal findings).

All participants had a rapid diagnostic test (RDT) for malaria performed (ICT Malaria Combo Cassette Test; ICT Diagnostics, Cape Town, South Africa; <http://www.ictdiagnostics.co.za/>). A blood slide for malaria thin- and thick-film examination and a 250- μL ethylenediaminetetraacetic acid (EDTA) tube for full blood cells count were collected from the same finger prick. If the RDT for malaria was negative, 6-mL of venous blood was collected for dengue serological analysis. All malaria-negative (assessed by RDT) patients were reviewed after at least 2 weeks and had a second 6-mL convalescent blood sample collected. If the RDT for malaria was positive, we considered malaria as the likely diagnosis, and venous blood was not drawn for DF screening.

Laboratory analysis. The 250- μL finger blood collection was stored in a fridge at 4°C , and a full blood cells count was performed within 4 hours using an automatic cell counter (COULTER ACT diff2). A threshold of $150 \times 10^9/\text{L}$ for platelets was used to define thrombocytopenia. This cutoff was chosen, because it is the usual value used by laboratories in clinical settings to define thrombocytopenia. All blood smears were stained with 2.5% buffered Giemsa (pH 7.2) for 35 minutes and examined by light microscopy. Slides were classified as negative if no parasites were seen in 100 thick-film fields by two different microscopists. The parasite species in positive films were identified, and densities were recorded as the number of parasites per 200 white blood cells (WBC). The results were used to confirm the presence or absence of malaria parasitemia in all subjects.

Venous blood samples were centrifuged, and the serum was stored at -80°C . Dry chemistry measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, and glucose was performed on serum using a strip test machine (Reflotron).

Acute serum samples were tested for the presence of dengue immunoglobulin G (IgG) antibodies and dengue NS1 antigen. Convalescent sera were tested for the presence of dengue IgG and IgM antibodies. PanBio Dengue Indirect IgG and Capture IgM tests were used and performed according to the manufacturer's recommendations. Dengue NS1 antigen was assessed using the BioRad Platelia Dengue NS1 antigen assay. Test results were classified as negative, equivocal, or positive if the sample to cutoff signal ratio was < 0.5 , $0.5\text{--}1.0$, or > 1.0 , respectively. Convalescent sera were also tested for Japanese Encephalitis (JE) IgM using PanBio combo test kits. Serology tests done on acute samples can be used for detecting both primary (i.e., IgM but no IgG) and secondary dengue (both IgG and IgM) infection.^{23–25} Prior exposure to a flavivirus was assumed if there was IgG detected in acute serum samples. NS1 antigen tests were used on acute samples to confirm acute dengue infection.

Case definition for DF and malaria. Interpretations of dengue serology (IgG and IgM) and NS1 results were as follows:

- Definite dengue: a positive NS1 or an IgG seroconversion with IgM detected in convalescent serum.
- Probable dengue: either an equivocal NS1 test in conjunction with an IgG seroconversion or dengue IgM in convalescent serum.
- Possible dengue: equivocal NS1 without other evidence of dengue or dengue IgG seroconversion without dengue IgM.
- Unlikely dengue: positive dengue IgG in acute serum with no other evidence of dengue.
- No dengue: no IgG seroconversion and negative convalescent IgG.
- Other flavivirus: IgG seroconversion with negative dengue IgM and negative NS1 or positive IgM for JE on combo assay.

Definite and probable cases were combined to define a clinical acute dengue infection (Figure 1). Patients with possible, unlikely, or no dengue results interpretations were considered as having another cause for their febrile illness. This classification was adopted first to be clinically meaningful and second to avoid overestimating the prevalence of DF.

A definite clinical malaria case (or malaria disease) was defined as fever $> 38^{\circ}\text{C}$ or history of fever with positive blood smear and/or positive RDT for malaria with negative test for dengue and absence of other obvious acute illness (like acute low respiratory tract infection or meningitis). A possible clinical malaria (versus alternative diagnosis) was defined as fever $> 38^{\circ}\text{C}$ or history of fever and positive RDT but negative blood slide in the absence of positive dengue case.

Data management and analysis. All data were entered using FoxPro software. Data analysis was performed using Excel and Stata (version 10.0) software. A Mantel–Haenszel test was used to calculate odds ratios (OR) in univariate analysis with 95% confidence intervals (CIs).

A *t* test was performed to investigate differences between means of continuous variables. A logistic stepwise logistic regression model was used to identify predictors of dengue versus non-dengue/non-malaria cases. Sex and age were included in this model in addition to all predictors identified in the univariate analysis (*P* value ≤ 0.1). An area under the

FIGURE 1. Classification of acute febrile illness (absolute numbers). RDT = rapid diagnostic test for malaria; BS = blood smear for malaria.

curve of receiver–operator characteristics (ROC) was calculated based on this regression model. We omitted IgG status of children below 1 year of age in stratified analysis to avoid possible interference with remaining circulating maternal antibodies.^{26,27}

RESULTS

Baseline characteristics. Five hundred seventy-eight patients presenting at the outpatient clinics of Yagaum rural hospital or Jomba Clinic with temperatures $> 38^{\circ}\text{C}$ or history of fever were enrolled in the study from October 2007 to June 2008; 48% were female, 75% were living in a rural area, and the mean age was 9.0 years (median = 5 years, range = 0–60 years).

There were 317 patients with a negative malaria test that were tested for dengue infection. Of these, 14% (45/317, 95% CI = 10–18%) were subsequently found positive for malaria on microscopy. Another 14% (45/317, 95% CI = 6–10%) of the patients presenting with fever were found positive for an acute dengue infection. Two hundred sixty-six samples with negative RDT were tested for NS1 antigens; 8% (21/266) were positive, and 10% (27/266) were equivocal. If the fever onset occurred less than 2 days previously, 12% (10/81) were NS1-positive. If it occurred 2–4 days before, 8% (3/40) were positive for NS1, and if it occurred 5 days or more before, 4% (3/77) were positive for NS1. For five NS1-positive tests, no onset date was recorded. The overall IgG seroprevalence for dengue antibody in acute samples was 83% (204/247, 95% CI = 78–87%). The seroprevalence of dengue IgG stratified by age group is shown in Figure 2.

Malaria was diagnosed in 52% (301/578, 95% CI = 48–56%) on the basis of a positive blood smear (BS). Twenty-six patients were RDT-positive for malaria, but BS was negative, and 10 patients whose tests were positive for dengue had a positive BS for malaria, making a total of 58% (337/578) of possible

FIGURE 2. Dengue IgG seroprevalence on acute samples; infant (< 1 year) seroprevalences were not shown because of existing maternal circulating antibodies.

and definite malaria cases. Complete demographic characteristics are shown in Table 1. The classification of malaria and dengue diagnoses according to tests done is shown in Figure 1. Median time between acute and convalescent sera was 18 days (interquartile range [IQR] = 15–29).

Clinical features. Clinical and laboratory features of all patients are presented in Table 2. The percentage of participants with platelet counts $< 150 \times 10^9/\text{L}$ in those with acute dengue and non-dengue/non-malaria was 46% (11/24, 95% CI = 25–66) and 47% (59/126, 95% CI = 38–56), respectively (OR = 0.98, $P = 0.9$, 95% CI = 0.63–1.52), whereas 84% (128/153, 95% CI = 78–90) of the participants with malaria had thrombocytopenia ($P < 0.001$). In a univariate analysis exploring the clinical features of DF compared with non-malaria/non-dengue cases, only facial flushing and sex (male) were found to be clinical predictors of dengue, with the following OR values: flushing face = 3.5 (95% CI = 1.2–10.3, $P = 0.02$) and sex (male) = 1.2 (95% CI = 1.0–1.4, $P = 0.02$). In a multivariate stepwise logistic regression model adjusting for major confounders (age, sex, headache, and skin eruption), we still found an association between dengue diagnosis and facial flushing (OR = 4.1, 95% CI = 1.4–12.4) and sex (OR = 1.2, 95% CI = 1.0–1.4). An area under the curve of the ROC for this regression model was 0.59.

Stratified analysis. The age-stratified proportions of dengue, malaria, and other illnesses are shown in Figure 3. A trend

FIGURE 3. Distribution across age groups of the prevalence of dengue, malaria, and other diseases in patients presenting with acute febrile illnesses in Madang.

TABLE 1

Baseline characteristics with prevalence of dengue and malaria infections

	Total numbers (%)	Acute dengue infection prevalence* (%)	Crude ORs	95% CI	Overall <i>P</i> values
Total patients	578	46 (8)			
Sex					
Female	276 (48)	18 (7)	1		
Male	282 (49)	24 (9)	1.3	0.7–2.5	0.37
Not stated	20 (3)	n/a			
Location					
Urban	142 (25)	16 (11)	1		
Rural	430 (75)	30 (7)	1.6	0.8–3.2	0.14
Age categories (in years)					
< 1	117 (20)	14 (12)	1		
1–4	168 (29)	16 (10)	0.8	0.4–1.7	
4–9	122 (21)	6 (5)	0.4	0.1–1.0	
10–14	59 (10)	3 (5)	0.4	0.1–1.4	0.03
15–25	43 (7)	3 (7)	0.6	0.1–2.0	
> 25	68 (12)	3 (4)	0.3	0.1–1.2	
Diagnosis of acute disease*					
Dengue	46 (8)	n/a			
Malaria	301 (52)	n/a			
Other	231 (40)	n/a			

Mean age = 9.0 years; median age = 5.0 years.

*Definite and probable cases of dengue (IgG seroconversion $\pm$ NS1-positive or equivocal $\pm$ IgM in convalescent serum); refer to the text for complete definitions.

for dengue acute infections to be more prevalent in children below 5 years ($P = 0.03$) was observed. Acute DF infections were more common in urban versus rural areas (11% versus 7%, respectively), but this did not reach significance (OR = 1.6, 95% CI = 0.8–3.2, $P = 0.14$). The IgG seroprevalence was higher in rural areas compared with urban areas: 86% versus 74% (OR = 0.5, 95% CI = 0.2–1.0, $P = 0.03$). Four participants had serological results compatible with other flavivirus infections; in one case, serology was consistent with infection by JE virus.

DISCUSSION

In the present study, we found that acute dengue infections are very common in outpatient settings in Madang, accounting for at least 8% of all febrile illnesses (14% of all non-malaria patients). This is an important finding, because PNG is not known to be endemic for dengue, and it has not previously been identified as a cause of febrile illnesses in the country. Dengue viral infections may contribute to the very high IgG flavivirus seroprevalence (by 10 years, almost the entire population has been exposed to a flavivirus), and it is likely that the virus has been circulating for a long time in this area. The role of other flavivirus infections to the overall IgG seroprevalence is not known, but the observation of higher seroprevalence in rural areas, where there may be less dengue transmission, suggests that other flaviviruses do, indeed, circulate in the area. Local clinicians were unaware of local dengue infections, suggesting that the disease is not recognized or is misdiagnosed as malaria. In neighboring countries, which are known to be endemic for DF, dengue seroprevalence is usually lower. For example, in the Solomon Islands, only 39% of the population (mainly adults) had IgG against dengue (versus 83% in PNG in our survey).^{28–30} In the age-stratified analysis, we observed a trend for symptomatic infections serologically confirmed to occur more frequently in young children. This probably relates to lower levels of pre-existing immunity. We also observed that the rate of positive NS1 was higher in the early course of the disease (less than 2 days).

The difference between the urban and rural rates of acute DF (11% versus 7%) did not reach statistical significance. We observed that acute malaria (all species) has the higher prevalence in children 5–9 years (79%), which is consistent with other observations in the same region (I. Mueller and others, personal communication). There was one participant whose serology was consistent with acute JE viral infection.

TABLE 2

Clinical and paraclinical features of patients

Clinical signs and symptoms	Dengue*	95% CI	Malaria†	95% CI	Other	95% CI	Overall <i>P</i> values
Duration of symptoms (days)	3.5	2.4–4.6	4.8	4.0–5.5	5	4.1–5.9	0.33
Chills (%)	38	23–53	68	62–73	43	36–49	< 0.01
Headache (%)	37	23–52	68	63–74	51	44–58	< 0.01
Eye pain (%)	47	20–73	47	39–54	64	27–45	0.02
Red face (%)	14	4–25	2	1–4	5	2–7	< 0.01
Myalgia (%)	23	10–36	31	25–36	28	22–34	0.54
Joint pain (%)	23	10–36	32	26–37	35	28–41	0.34
Skin eruption (%)	5	0–12	n/a	n/a	1	1–2	n/a
Bleeding signs (%)	n/a	n/a	1	1–3	0	0–1	n/a
Adenopathies (%)	5	0–11	2	1–4	1	0–2	n/a
Cough (%)	58	43–73	43	37–49	68	62–75	< 0.01
Dyspnoea (%)	14	4–25	6	3–9	15	11–20	< 0.01
Diarrhea (%)	7	0–15	10	7–14	12	8–16	0.55
Vomiting (%)	15	4–26	38	32–43	26	20–32	< 0.01
Taste modification (%)	7	0–15	21	17–26	16	11–21	0.04
Paraclinical parameters							
Glucose (mmol/L)	6.9	4.4–9.5	7	6.3–7.8	9.6	4.0–15.2	0.3
AST (μ L/L)	41	27–55	52	10–95	33	28–38	0.3
ALT (μ L/L)	21	13–30	31	0–64	18	15–21	0.4
Creatinin (mmol/L)	51	44–58	56	51–61	61	54–68	0.3
Leukocytes ($\times 10^9$ /L)	10.4	6.7–14.1	6.3	5.8–6.8	9.2	8.1–10.4	< 0.01
Hemoglobin (g/dL)	84	73–96	74	69–78	88	84–93	< 0.01
Hematocrit (%)	27	24–31	25	24–26	29	28–31	< 0.01
Platelets ($\times 10^9$ /L)	191	136–245	97	86–108	175	156–193	< 0.01
Rate of plt $< 150 \times 10^9$ /L (%)	46	25–66	84	78–90	47	38–56	< 0.01

*Definite and probable cases of dengue (IgG seroconversion $\pm$ NS1-positive or equivocal $\pm$ IgM in convalescent serum); refer to the text for complete definitions.

†Clinical malaria confirmed by positive blood slide.

There has only been one other case of JE identified in humans in PNG, but there is evidence of its presence in mosquito populations.^{31,32}

The clinical features of acute DF episodes in our cohort were not specific, and we did not observe the typical clinical picture of rash, eye pain, headache, and fever. In our study, dengue was mostly an undifferentiated febrile illness, which is confirmed by the multivariate analysis; it was unable, however, to identify clinical predictors of dengue cases compared with non-dengue/non-malaria cases, except facial flushing and sex (male). The platelets count was also not lower in the DF cases compared with non-dengue/non-malaria patients (rate of thrombocytopenia $< 150 \times 10^9/L = 46\%$ versus 47%). This observation is important, because thrombocytopenia is often described with DF and is a typical feature of DHF.^{33–35} As expected, almost all malaria cases had a thrombocytopenia (84%). These findings contrast with recent work in Cuba (where there is no malaria), highlighting the use of thrombocytopenia as a predictor of dengue.³⁶

None of the patients with DF developed severe forms of the disease (DHF or DSS). Anecdotally, hemorrhagic forms of DF have not been observed by clinicians working in hospitals in the study area. The observation that DHF is very rare or absent in Madang, despite a very high prevalence of acute dengue infections, is important. Mechanisms leading to hemorrhagic complications in DF are not clearly understood, and many factors have been identified to increase the risk of developing DHF like urbanization, multiple serotype cocirculation, or host genetic factors. One study performed in Haiti³⁷ made similar observations and postulated that the African origins of the population might provide some protection against bleeding complications. More generally, this theory is often mentioned to explain the absence of DHF in Africa.³⁸ In the present study, we have a population with a different genetic background. Whether Melanesian populations are at less risk of DHF should be further investigated. Other factors, including the viral subtypes circulating or density of vector populations, may explain the epidemiology observed. Past experience does not preclude the possibility that PNG will experience outbreaks of DHF in the future.

The present study has some limitations. First, the relatively small number of DF cases did not allow us to perform more detailed analysis on the pattern of the disease. Second, because the project is located only in one region of the country, we cannot make conclusions about the national dengue epidemiology. We believe, however, that it is likely that dengue will be found elsewhere in the country, because the vectors (*Ae. aegypti* and *Ae. albopictus*) are present all over the country, especially in the coastal areas.³⁹ Third, it is possible that the incidence of acute dengue infections has been underestimated by our testing strategy: patients with positive malaria RDTs did not undergo dengue testing, and we only included cases with definite or probable serology/antigen testing. We are well aware that coinfections with malaria and dengue are possible, and we may have missed DF cases among the malaria cases. The purpose of the study, however, was to document the occurrence of dengue rather than to estimate the incidence, which would likely vary with geography and time of year. Resources were limited, and we focused our attention on patients with negative RDTs, a group that we believed was more likely to suffer from DF or other infections. The conservative approach means that the rate of 8% of acute DF is the lowest estimate.

In conclusion, acute symptomatic dengue infections are common in the Madang Province; this is an important finding, because only a few epidemics have been reported in PNG in the past. The clinical presentation is non-specific and difficult to recognize by health workers. Thrombocytopenia was observed more commonly with malaria and could not be used to differentiate dengue from the non-malaria/non-dengue groups. DHF was not observed for reasons that are unknown. It is important that a more extensive understanding of the epidemiology of dengue in PNG is gained to better estimate the potential risk of epidemics of DHF in the future.

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