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Short Report: Prevalence of Patients with Acute Febrile Illnesses and Positive Dengue NS1 Tests in a Tertiary Hospital in Papua New Guinea

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Abstract. Because the prevalence of dengue fever in urban settings in Papua New Guinea is unknown, we investigated the presence of dengue using the NS1 antigen test in an outpatient-based prospective observational study at Port Moresby General Hospital. Of 140 patients with acute febrile illnesses, dengue fever was diagnosed in 14.9% (20 of 134; 95% confidence interval [95% CI] = 9.6–22.4). Malaria (2 of 137; 1.5%; 95% CI = 0.3–5.7), chikungunya (3 of 140; 2.1%; 95% CI = 0.6–6.6), and bacterial bloodstream infections (0 of 80; 0%; 95% CI = 0–5.7) were uncommon. Dengue fever should no longer be considered rare in Papua New Guinea.

Globally, an estimated 50–100 million cases of dengue fever (DF) and hundreds of thousands of dengue hemorrhagic fever cases occur annually.[1] In the Asia Pacific region, dengue is recognized as an emerging vector-borne disease.[2] However, despite the predominance of dengue virus and vectors in this region, DF is rarely diagnosed in Papua New Guinea (PNG), and dengue hemorrhagic fever has not been reported apart from an unconfirmed outbreak more than a decade ago.[3] Nevertheless, this may be a reflection of (1) the lack of viral diagnostic facilities in PNG, (2) the fact that previous dengue investigations have been conducted in predominantly rural populations,[3–5] and (3) the low index of clinical suspicion among health workers in PNG.

Because the prevalence of DF in urban PNG is unknown, we hypothesized that it may be an important cause of acute febrile illnesses and conducted a prospective observational study at Port Moresby General Hospital, the main tertiary hospital in PNG, between February and August of 2013. Patients were recruited through the Adult's and Children's Outpatient Departments. Children were defined as age ≤ 14 years. Study inclusion criteria included (1) an axillary temperature ≥ 38°C or a history of fever in the past 5 days, (2) clinical signs and symptoms suggestive of an acute febrile illness, and (3) a signed informed consent by the patient or a guardian. Those who did not fulfill the inclusion criteria and infants age < 1 month were excluded. Demographic data, relevant clinical signs, symptoms, and basic clinical assessments were recorded on standardized case report forms by a study clinician (V.A.). Ethical approval was obtained from the University of Papua New Guinea School of Medicine and Health Sciences Ethics Committee and the Medical Research Advisory Committee of PNG.

A diagnosis of dengue was based on the serological detection of NS1 antigens. The Bio-Rad Platellia Dengue NS1 antigen assay was used, and results were classified as negative, equivocal, or positive based on a signal ratio of < 0.5, 0.5–1.0, or > 1.0, respectively. A diagnosis of malaria was based on microscopic examination of thick blood smears. Chikungunya was investigated by use of an immunoglobulin M (IgM) antibody immunochromatographic rapid diagnostic test (BIOLINE, Kyonggido, Korea) with polymerase chain reaction confirmation in positive cases as part of an outbreak investigation.[6] Biochemistry parameters were analyzed using a Vitros 250 chemistry analyzer, whereas full blood counts were performed using an automated Sysmex XT1800i hematology analyzer. Blood (4–5 mL) was collected for culture in Trypticase Soya Broth media, which cover a wide spectrum of organisms, that were prepared in house with manual workup for 72 hours. Statistical analysis was performed using STATA 11.0 (Stata Corp., College Station, TX). The Mann–Whitney test was used for comparisons between groups for continuous variables, and the two-sided Fisher's exact test was used for comparisons between proportions.

Of 140 patients with acute febrile illnesses screened, none refused consent, 57.1% were males, and 38.6% were children. The median age was 6 (interquartile range = 3–8) years in children and 33 (interquartile range = 25–39) years in adults. In six patients, dengue could not be investigated because of inadequate samples. Of those patients tested, DF was equivocal and considered a possible diagnosis in 3 patients and positive in 20 patients (20 of 134; 14.9%; 95% CI [95% CI] = 9.6–22.4). Clinical and laboratory features of these patients combined are shown in Table 1. Most (73.9%) were urban residents (residing within the city of Port Moresby), with no particular suburb showing a greater proportion of DF cases. Vomiting was significantly more common in children with a positive NS1 test compared with those with a negative test (66.7% versus 20.9%; $P = 0.012$). In adults, chills and rigors (92.3% versus 58.3%; $P = 0.025$) and myalgia (100% versus 67.8%; $P = 0.015$) were significant clinical features of DF.

Malaria (2 of 137; 1.5%; 95% CI = 0.3–5.7), chikungunya (3 of 140; 2.1%; 95% CI = 0.6–6.6), and bacterial bloodstream infections (0 of 80; 0%; 95% CI = 0–5.7) were uncommon. A pathogen could not be identified in 80.0% (112 of 140; 95% CI = 72.2–86.1) of patients. Significantly higher rates of thrombocytopenia, neutropenia, monocytosis, and eosinopenia were observed in patients with DF (Table 1). There was evidence of hemoconcentration in significantly more patients with DF with hemoglobin levels ≥ 15 g/dL (19.1% versus 4.9%; $P = 0.045$), resulting in a significant difference in median hemoglobin and pack cell volume observed between the two groups (Table 1).

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TABLE 1
Baseline demographic, clinical, and laboratory characteristics of study participants

	Dengue NS1 test positive ($N = 23$)	Dengue NS1 test negative ($N = 111$)	P value
Male sex	15/23 (65.2)	61/111 (55.0)	0.49
Children	9/23 (39.1)	43/111 (38.7)	> 0.99
Chills and rigors	18/23 (78.3)	58/103 (56.3)	0.06
Vomiting	11/ 23 (47.8)	23/104 (22.1)	0.02
Headache	20/23 (87.0)	70/98 (71.4)	0.18
Flushing of the face	16/23 (69.6)	57/101 (56.4)	0.35
Eye redness	11/23 (47.8)	52/103 (50.5)	> 0.99
Rash	2/23 (8.7)	16/100 (16.0)	0.52
Dark urine	0/23 (0.0)	1/103 (1.0)	> 0.99
Diarrhea	4/23 (17.4)	15/100 (15.0)	0.75
Enlarged cervical glands	1/23 (4.3)	13/100 (13.0)	0.47
Myalgia	18/23 (78.3)	65/94 (69.1)	0.45
Arthralgia	18/23 (78.3)	71/94 (75.5)	> 0.99
Temperature (°C)	38.3 [38.0–38.6]	38.3 [37.6–39.0]	0.94
Hemoglobin (g/dL)	13.8 [11.9–14.5]	12.2 [11.0–13.4]	0.01
Packed cell volume	40.7 [35.0–44.0]	37.0 [33.5–41.0]	0.02
Mean cell volume	81.0 [76.0–83.0]	78.0 [70.9–83.0]	0.36
Mean cell hemoglobin	28.0 [26.0–31.0]	26.3 [24.0–29.0]	0.06
Mean cell hemoglobin concentration	33.4 [32.0–34.0]	32.2 [31.0–33.7]	0.22
Red blood cells	5.0 [4.8–5.4]	4.8 [4.4–5.4]	0.27
Platelets ($\times 10^9$/L)	156.0 [73.0–205.0]	212.0 [162.0–268.0]	0.002
Thrombocytopenic (platelets < 100 $\times$ 10^9/L)	7/21 (33.3)	9/101 (8.9)	0.01
Total white blood cells ($\times 10^9$/L)	5.3 [4.7–9.6]	7.7 [5.7–10.8]	0.06
Neutrophils (%)	52.0 [34.0–71.9]	65.0 [57.0–76.0]	0.02
Lymphocytes (%)	37.0 [15.0–45.0]	23.0 [13.0–33.0]	0.08
Monocytes (%)	13.0 [9.0–16.0]	9.0 [6.0–11.0]	0.002
Eosinophils (%)	0.8 [0.0–1.3]	1.8 [0.4–4.0]	0.008
Urea (mmol/L)	4.9 [3.5–5.7]	3.8 [2.8–5.1]	0.06
Creatinine (mmol/L)	80.0 [44.0–106.5]	67.0 [45.0–93.0]	0.51
Sodium (mmol/L)	137.0 [134.0–140.0]	140.0 [138.0–142.0]	0.01
Potassium (mmol/L)	4.1 [3.7–4.5]	4.0 [3.7–4.3]	0.44
Chloride (mmol/L)	104.0 [102.0–106.0]	106.0 [104–109]	0.04
AST (U/L)	75.0 [36.0–129.5]	42.0 [31.0–66.0]	0.09
Alanine aminotransferase (U/L)	40.0 [28.5–66.0]	37.0 [25.0–55.0]	0.64
Alkaline phosphatase (U/L)	104.5 [75.5–203.5]	111.0 [76.0–213.0]	0.58
Total protein (g/L)	79.5 [72.5–83.0]	77.0 [72.0–81.0]	0.35
Albumin (g/L)	38.0 [34.0–42.0]	40.0 [36.0–43.0]	0.25
Positive blood culture	0/14 (0)	0/66 (0)	–

Data are numbers (percentages) or medians [interquartile ranges].

A high proportion of patients with DF had elevated aspartate aminotransferase (AST; 62.5% versus 36.7%; $P = 0.07$). Other laboratory parameters were mostly within normal PNG limits,[7] and observed differences did not seem to have clinical significance.

Using the NS1 test, this study is the first from an urban setting in PNG to investigate DF in patients with acute febrile illnesses. We showed a high prevalence rate of 14.9% compared with 8% in a previous report from the north coast of PNG that used the NS1 and serology tests in paired samples.[4] In addition, malaria, which is often considered endemic in PNG, contributed only 1.5% of cases, whereas chikungunya accounted for 2.1% of cases. Findings from this study support our hypothesis that a lack of viral diagnostic facilities compounded by the lack of dengue baseline prevalence data and the low index of clinical suspicion by health workers may have contributed to an underestimation of the true burden of DF in PNG. This underestimation continues to place PNG in a vulnerable position with regards to the risk of potential outbreaks and the awareness of dengue as an emerging disease.

Although malaria was the most common clinical diagnosis in patients in our study, these data do not support malaria as an important cause of acute febrile illnesses in Port Moresby. Our findings are supported by studies from malaria-endemic areas that strongly suggest that, with the global decline in malaria, viral pathogens may become important etiological causes of acute febrile illnesses.[5,8] This has important clinical implications for PNG, where most patients will not benefit from empiric use of antibiotics or antimalarial drugs, therefore increasing the need for accurate diagnostics.

Our study had limitations. First, we could not exclude bacterial causes of acute febrile illnesses in 39% of patients with a positive NS1 test and 44% of patients with a negative test (Table 1), and the manual blood culture system used in this study may not be optimal to detect bacteremia. However, the fact that the majority of our patients were outpatient cases and the low incidence of bacterial bloodstream infection that has been reported, even in severely ill PNG patients, when automated blood culture systems are used[9,10] highly suggest that bacterial infection was not present. Second, serotyping of dengue viruses and confirmatory diagnosis using more sensitive molecular methods were not possible. Although the dengue NS1 antigen test proved to be a cost-effective diagnostic tool for our setting that can be used under field conditions, which was shown in this study as well as in a previous study,[4] additional validation studies comparing its use with more

sensitive diagnostic methods are needed. Third, we were unable to further identify pathogens in 80% of our patients.

These data suggest that DF should no longer be considered uncommon in PNG patients with acute febrile illnesses, particularly in urban areas. Because extravasation is a feature of both dengue hemorrhagic fever and DF, the presence of hemoconcentration in some of our patients with DF may also suggest the possibility of unrecognized dengue hemorrhagic disease. Additional studies will be needed to investigate this observation as well as the seasonality of DF in PNG. Although most patients with acute febrile illnesses may not benefit from antibiotics and antimalarial drugs, the high rate of unidentified pathogens further highlights the need for accurate diagnostics that can guide the rational use of antimicrobial drugs in PNG.

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