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Abstract

Background: Dengue virus infection is spreading globally and most parts of Sudan have witnessed repeated dengue outbreaks, with the detection of DENV-1, DENV-2 and DENV-3 serotypes.

Aims: In this report we describe the dengue fever outbreaks that occurred in eastern Sudan (Kassala and Port Sudan cities) from August to November 2019.

Methods: We enrolled 79 (29.8%) suspected cases from Kassala and 186 (70.2%) from Port Sudan who presented with fever. The participants were medically examined and their clinical signs recorded. Blood samples were collected for complete blood count, detection of anti-dengue virus IgM, detection of NS1 dengue antigen and identification of the virus serotype using RT-PCR.

Results: The main clinical presentations were fever, abdominal pain, joint pain and vomiting, and thrombocytopenia was the main laboratory finding. One hundred and twenty-five blood samples tested positive for the anti-dengue IgM antibody, and 145 were positive for the NS1 antigen. Using RT-PCR, we identified 35 (24%) infections with DENV-2, 100 (69%) with DENV-3 and 10 (7%) with DENV-4 serotypes.

Conclusions: We identified multiple serotypes – DENV-2, DENV-3 and DENV-4 – as the causes of the outbreak. The presence of DENV-4 serotype was documented for the first time in Sudan.

Keywords: dengue fever, outbreak, serotypes, DENV-4, Sudan

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Introduction

Dengue fever (DENG) is an expanding global health problem. It is caused by mosquito-transmitted Dengue virus (DENV), a member of the genus Flavivirus, family Flaviviridae. Four serotypes of DENV cause 390 million annual infections worldwide, with 240

million remaining asymptomatic maintaining the transmission of the virus (1,2).

Dengue virus serotypes have been detected in a number of outbreaks in several countries in Africa and the Middle East (3–6) and dengue outbreaks have repeatedly occurred in east African countries, in Yemen and Saudi Arabia (6–9).

Sudan has witnessed repeated outbreaks of dengue during the last 2 decades. Outbreaks of dengue haemorrhagic fever were reported in an outbreak in Port Sudan in 2005, with a 3.8% mortality. The outbreak was caused by DENV-3 (serotype 3) and affected mostly young individuals aged 5–15 years. Another outbreak occurred in Port Sudan in 2010, with a total of 3765 cases (10).

Dengue outbreaks were reported in Kassala State, eastern Sudan, on the border with Eritrea, between March 2016 and March 2017 (11). The outbreak extended south-west to El-Gadaref State. Dengue outbreaks were reported in El Obeid in North Kordofan in December 2012 and January 2013 (12). Darfur states in western Sudan have also witnessed several dengue outbreaks (13,14).

Three DENV serotypes, DENV-1, DENV-2 and DENV-3, have previously been detected in outbreaks in Sudan (15,16).

In this study we describe an outbreak of dengue in the cities of Kassala and Port Sudan in eastern Sudan where 3 dengue serotypes, DENV-2, DENV-3 and DENV-4, were detected.

Methods

Ethical considerations

The study was approved by the Institute Review Board at Alzaeem Alazhari University, Sudan and the ethics committee of Kassala State Ministry of Health. Consent was obtained from the participants/parents or guardians before enrolment in the study.

Study sites

The study was conducted in eastern Sudan in the cities of Kassala and Port Sudan. Kassala is

close to the border with Eritrea while Port Sudan is on the Red Sea coast. Kassala has a dry climate with seasonal rains during June–October. The city suffers from floods and accumulation of water in ponds during the rainy season. Port Sudan has a hot and humid summer. Due to water shortages in the city, water is stored in-house in domestic containers.

Enrolment of participants

An increased number of cases of fever were passively reported to health centres and hospitals in Kassala and Port Sudan cities during the period August–November 2019. Based on the protocol of the Federal Ministry of Health for diagnosis of fever, all patients were tested for malaria using blood film and rapid diagnostic tests, and positive cases were treated with antimalaria drugs according to the national malaria case management protocol.

Due to the lack of response to antimalaria treatment and reports of a few cases of haemorrhage, dengue fever was suspected and investigated. Patients reporting with fever during the period August–November 2019 were consecutively enrolled following their, or their parents' or guardians' consent. We enrolled 79 participants from the paediatric hospital in Kassala and 186 from Port Sudan Hospital.

Clinical data

The participants were clinically examined and clinical findings and demographic data were recorded.

Venous blood samples (2 mL) were collected from each participant in ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count, differential white blood cell count, platelet count, haemoglobin measurement and ribonucleic acid (RNA) extraction from the buffy coat for identification of the virus serotype. We collected 2 mL of blood in plain tubes for serum separation and detection of anti-dengue virus IgM/IgG and detection of DENV-NS1 antigen

Haematological analysis

Total white blood cell counts, differential white blood cells, haemoglobin and platelet counts were carried out using a Sysmex haematology analyser.

Detection of anti-dengue IgM and NS1 antigen

We screened 265 serum samples to measure anti-dengue virus IgM/IgG antibody using Euroimmun ELISA (Euroimmun AG, Lübeck, Germany) following the procedure recommended by the manufacturers. A positive reaction was determined by comparison with IgM/IgG positive reference sera provided by the manufacturer (using the cut-off OD > 0.3, according to the supplier).

Dengue NS1Ag was detected in plasma samples using Platelia Dengue NS1 Ag-ELISA (Biorad Laboratories, Marnes-la-Coquette, France) following the procedure recommended by the supplier. The optical density reading was measured using a microtitre plate reader at 450–620 nm (DIASource ELISA Reader, DIAS2000, Belgium). The presence of NS1 antigen in plasma samples was determined by comparing the optical densities of the samples and that of the control serum.

Identification of dengue serotypes

We used the Easy BLUETM Total RNA Extraction kit (Intron Biotechnology, Seoul South Korea) for extraction of RNA from the buffy coat layer, which was frozen at –80° C following the instruction of the supplier. The RNA concentration and purity were measured using a Nanodrop spectrophotometer at 260 nm.

Dengue serotype specific primers designed and validated by Nguyen (DENV-1F, DENV-1R; DENV-2F, DENV-2R; DENV-3F, DENV-3R; DENV-4F, DENV-4R) (Table 1) were used in the multiplex RT-PCR assay as described by Nguyen (17).

Reverse transcription of the extracted virus RNA was performed using OneStep RT-PCR Premix (Intron Biotechnology, South Korea) with a final volume of 20 uL using 7 uL of the extracted RNA. The following PCR cycles were used: 45°C for 30 min and 94ºC for 5 min, followed by 45 amplification cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min, with a final extension at 72°C for 10 minutes using a thermal cycler (PRIMUS 96, Gradient Peglab, Germany). The amplified products were analysed by electrophoresis on a 3% agarose gel containing 1.5 µg/mL ethidium bromide for ultraviolet visualization.

Statistical analysis

Descriptive statistics were used for the serology results, the clinical signs and the laboratory variables.

Results

Participants

Among the 265 participants, 182 (68.67%) were males and 83 (31.33%) were females. Age ranged between 0.5 and 65 years, with a mean of 15 years.

Signs and symptoms

Severe clinical signs were seen in 21 patients (splenomegaly, and/or hepatomegaly, and/or lymph-node enlargement); 19 patients had bleeding tendency (epistaxis + haematemesis) and 72 had vomiting; 8 patients died (Table 2). The laboratory investigations identified leukopenia and pancytopenia. A significant correlation was determined between detection of anti-dengue IgM and leukopenia and IgM and pancytopenia.

One hundred and twenty-five patients tested positive for anti-dengue IgM antibodies while 16 were equivocal; 72.0% of the IgM-positive cases had thrombocytopenia 5days (Table 3). The haemoglobin levels were