Detection of dengue virus serotype 4 in Sudan

Mohamed Desogi,[1] Mostafa Ali,[2,3] Nesrin Gindeel,[2] Fatima Khalid,[4] Muhannad Abdelraheem,[5] Aymen Alnaby,[2] Mariam Saad,[2,3] Elwaleed Elamin,[2,3] Musa Kheir[6] and Maowia Mukhtar[2,7]

[1]Department of Paediatrics and [4]Department of Biochemistry, Faculty of Medicine and Health Sciences, University of Kassala, Kassala, Sudan. [2]Bioscience Research Institute, Ibn Sina University, Khartoum, Sudan (Correspondence to Maowia M. Mukhtar: mmukhtar@tropedicine.org). [3]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Alzaeem Alazhari University, Khartoum, Sudan. [5]Ministry of Health, Port Sudan, Sudan. [6]Faculty of Medicine and [7]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

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 94°C 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.

Table 1 Nucleotide sequence of primers used for the identification of dengue serotypes among patients with fever in eastern Sudan, 2019

Serotype	Primer sequences (5–3)	Position	Product size in base
DENV-1F	ATCCATGCCCATCACCAAT	9865–9883	100
DENV-1R	TGTGGGTTTTGTCCTCCATC	9945–9964	
DENV-2F	TCCATACACGCCAAACATGAA	9859–9879	125
DENV-2R	GGGATTTCCTCCCATGATTCC	9963–9983	
DENV-3F	TTTCTGCTCCCACCACTTTC	9591–9610	118
DENV-3R	CCATCCYGCTCCTTGAGA	9691–9708	
DENV-4F	GYGTGGTGAAGCCYCTRGAT	9587–9607	178
DENV-4R	AGTGARCGGCCATCCTTCAT	9744–9764	

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 < 100 000 cells/mm^3 and presented with fever for > 5days (Table 3). The haemoglobin levels were < 13.7 mg/dL in 98 (78.4%) patients, and 53 (42.4%) had white blood cells counts < 3900 cells/mm^3.

Detection of anti-dengue IgM antibody

Detectable NS1 antigen was found in 145 patients, while 27 were equivocal. All samples that tested positive for NS1 antigen had thrombocytopenia < 150 000 cells/mm^3 and presented with fever for < 4 days. RT-PCR serotyping of NS1-positive samples detected DENV-2 (125 bp) in 35 (24%), DENV-3 (118 bp) in 100 (69%) and DENV-4 (178 bp) in 10 (7%) of the blood samples collected from patients during the outbreaks (Figure 1). Malaria co-infection was detected in 14.9% of the patients diagnosed with dengue.

Table 2 Frequency of clinical signs and outcome of dengue infection among patients with fever in Kassala and Port Sudan cities, 2019

Clinical sign	No.	%
Fever	265	100
Joint pain	74	28
Abdominal pain	74	28
Vomiting	72	27
Cough	72	27
Diarrhoea	24	9
Bleeding tendency	19	7
Convulsion	13	5
Splenomegaly	11	4
Skin rash	8	3
Itching	8	3
Hepatomegaly	8	3
Enlargement of lymph nodes	5	2
Death	8	3

Table 3 Haematology results for patients with fever who tested positive for anti-dengue IgM antibodies (*n* = 125), eastern Sudan, 2019

Investigation	No.	%
Total white blood cells Leukopenia < 3900 cells/mm^3	53	42.4
Haemoglobin < 13.7 mg/dL	98	78.4
Thrombocytopenia < 100 000/mm^3	90	72.0

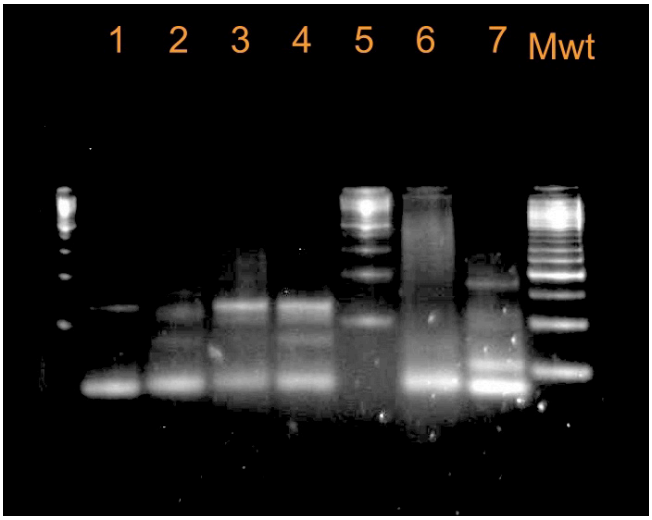
Discussion

Dengue fever is an increasing health problem globally (*18*). Repeated dengue outbreaks have occurred in many parts of Sudan (*12,13,19,20*). Previous reports on dengue in Sudan described localized outbreaks based on clinical signs and serology tests.

This 2019 dengue outbreak occurred in Kassala city and Port Sudan in eastern Sudan. The vector control measures in the 2 sites had been disrupted during previous years. Dengue virus infection was suspected following a sudden increase in passively reported cases of fever among residents of the 2 cities. The suspected patients were initially investigated for malaria according to the national protocol for diagnosis of fever as malaria is endemic in the region (*19,21*). Many patients were treated for malaria regardless of the results of the diagnosis, and before enrolment in our study. Such decisions are common and reflect the lack of confidence in malaria diagnosis. Dengue fever was suspected based on previous history of dengue outbreaks in the 2 cities (*19,21*). Interestingly, the male:female ratio among patients enrolled from Kassala was 1:1, differing from the Port Sudan ratio of 2:1, reflecting the exposure of more males in the sample from Port Sudan. This could be explained by the fact that most of the participants from Port Sudan were inmates from the main adult male prison in the city. The most frequent clinical signs were fever, abdominal pain, joint pain and vomiting, similar to the findings described in previous studies of dengue outbreaks in eastern Sudan (*19*). The laboratory investigations of the participants were marked by leukopenia and pancytopenia, similar to previous reports in dengue patients (*19*).

Dengue infection was confirmed by detection of anti-dengue IgM antibodies and detection of the dengue NS1 antigen. Confirmation had been delayed because malaria was suspected first. Patients who tested positive for the dengue NS1 antigen had thrombocytopenia as a hallmark, and the test identified early infection among patients who had had fever for < 4 days. The higher sensitivity of NS1 antigen detection over anti-dengue IgM ELISA was evident because all IgM positive patients were positive on the NS1 antigen detection test. Similar findings have

Figure 1 Amplified PCR products of dengue virus serotypes (125bp- DENV-2, 118bp- DENV-3 and 178bp- DENV-4), eastern Sudan, 2019 [lanes 1 and 2 DENV-3 (118bp); lanes 3 and 4 DENV-2 (125bp); lanes 5 and 8 Mwt marker 50bp; lane 6 negative control; lane 7 DENV-4 (178bp)]



been reported previously (*22,23*). The samples which tested negative in anti-dengue IgM serology and NS1 antigen tests should have been further investigated for infection by other arboviruses since Chikungunya and Rift Valley virus infections have previously been reported from the 2 study sites (*24*).

Reverse transcriptase PCR using dengue serotype specific primers identified multiple DENV-2, DENV-3 and DENV-4 serotypes as the causes of the outbreaks based on the PCR amplification of the samples that generated characteristic PCR fragment sizes: 125bp (DENV-2), 118bp DENV-3 and 178bp DENV-4) (*17*). To our knowledge, this is the first detection of the DENV-4 serotype in Sudan (*16*). DENV-4 prevalence is less reported globally, but has been reported in West Africa, Brazil and South East Asia (*25*). The geographic location of Port Sudan on the Red Sea is significant: it is used for the import of commodities to several African countries, including South Sudan, Chad and the Central African Republic, and the movement of individuals between Sudan and the countries around the Persian Gulf could result in the spread of DENV-4 to these countries.

Our findings are significant and warrant further investigation of possible malaria–arbovirus co-infection during outbreaks. We recommend the consideration of dengue fever in the differential diagnosis of patients presenting with fever in Sudan, and emphasize the need for screening of travellers to minimize the introduction of new strains of dengue and other arboviruses to the country. These measure will also help reduce the incidence of malaria, which is prevalent in the area.

The views expressed in this publication are those of the authors and not necessarily those of the National Institute for Health Research or the Department of Health and Social Care.

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Competing interests: None declared.

Détection du sérotype 4 du virus de la dengue au Soudan

Résumé

Contexte : L'infection par le virus de la dengue se propage dans le monde entier. La plupart des régions du Soudan ont connu des flambées récurrentes de dengue, marquées par la détection des sérotypes DENV-1, DENV-2 et DENV-3.

Objectifs : Le présent rapport fait état des flambées de dengue survenues dans l'est du Soudan (dans les villes de Kassala et de Port-Soudan) entre août et novembre 2019.

Méthodes : Nous avons inscrit 79 cas suspects à Kassala (29,8 %) et 186 à Port-Soudan (70,2 %) qui présentaient des symptômes de fièvre. Les participants ont fait l'objet d'un examen médical et les signes cliniques qu'ils affichaient ont été enregistrés. Des échantillons sanguins ont été prélevés pour la réalisation d'une numération complète de la formule sanguine, la détection des anticorps IgM dirigés contre le virus de la dengue, la détection de l'antigène NS1 de la dengue et l'identification du sérotype viral par RT-PCR.

Résultats : Les principaux tableaux cliniques présentés incluaient de la fièvre, des douleurs abdominales, des douleurs articulaires et des vomissements. La thrombocytopénie constituait le principal résultat biologique.

Cent vingt-cinq échantillons sanguins présentaient des résultats positifs à la recherche de l'anticorps IgM contre la dengue, et 145 se sont révélés positifs à la recherche de l'antigène NS1. À l'aide de la technique RT-PCR, nous avons identifié 35 infections par le DENV-2 (24 %), 100 infections par le DENV-3 (69 %) et 10 infections par des sérotypes DENV-4 (7 %).

Conclusions : Nous avons identifié plusieurs sérotypes (DENV-2, DENV-3 et DENV-4) comme causes de la flambée de dengue. La présence du sérotype DENV-4 a été documentée pour la première fois au Soudan.

اكتشاف النمط المصلي 4 لفيروس حمى الضنك في السودان

محمد دسوقي، مصطفى علي، نسرين قنديل، فاطمة خالد، مهند عبد الرحيم، أيمن النبي، مريم سعد، الوليد الأمين، موسى خير، معاوية مختار

الخلاصة

الخلفية: تنتشر عدوى فيروس الضنك على المستوى العالمي. وشهدت معظم أنحاء السودان فاشيات متكررة لحمى الضنك، مع اكتشاف الأنماط المصلية لحمى الضنك، وهي: DENV-1، و DENV-2، و DENV-3.

الأهداف: هدفت هذه الدراسة الى وصف فاشيات حمى الضنك التي حدثت في شرق السودان (مدينتي كسلا وبورتسودان) من أغسطس/ آب إلى نوفمبر/ تشرين الثاني 2019.

طرق البحث: بلغ عدد الحالات المُشتبه فيها التي ظهرت عليها أعراض الحمى وسجلناها 79 حالةً (29.8٪) من كسلا، و186 حالةً (70.2٪) من بورتسودان. وفُحص المشاركون طبيًّا، وسُجلت علاماتهم السريرية. وجُمعت عينات الدم لإجراء تعداد كامل لعناصر الدم، والكشف عن مضادات مجموعة الجلوبولين المناعي لفيروس حمى الضنك، والكشف عن مستضد البروتين اللابنيوي لفيروس حمى الضنك، وتحديد النمط المصلي للفيروس باستخدام التناسخ العكسي لتفاعل البوليميراز المتسلسل.

النتائج: تمثلت الأعراض السريرية الرئيسية في الحمى، وألم البطن، وألم المفاصل، والقيء، وقلة الصُّفَيحات، وذلك وفقًا للنتائج المختبرية الرئيسية. وجاءت نتيجة اختبار مائة وخمس وعشرين عينة دم إيجابية لمضادات مجموعة الجلوبولين المناعي لفيروس حمى الضنك، فيما جاءت نتيجة اختبار 145 عينةً إيجابية لمستضد البروتين اللابنيوي. وباستخدام التناسخ العكسي لتفاعل البوليميراز المتسلسل، حددنا 35 حالة عدوى بالنمط المصلي DENV-2 (24٪)، و100 حالة إصابة بالنمط المصلي DENV-3 (69٪)، و10 حالات إصابة بالنمط المصلي DENV-4 (7٪).

الاستنتاجات: حددنا أنماطًا مصلية متعددة DENV-2، وDENV-3، وDENV-4 باعتبارها أسباب الفاشية. ووُثِّق وجود النمط المصلي DENV-4 لأول مرة في السودان.

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