Am. J. Trop. Med. Hyg., 98(5), 2018, pp. 1469–1471
doi:10.4269/ajtmh.17-0634

Dengue Fever as an Emerging Infection in Southeast Iran

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Abstract. Dengue fever (DF) is a mosquito-borne acute viral disease presenting with hemorrhagic manifestations in severe cases. Southeast Iran is in close proximity to Pakistan, an endemic country for DF. This cross-sectional study was conducted in the Sistan and Baluchestan province in the southeast of Iran to investigate possibility of DF (immunoglobulin M [IgM], immunoglobulin G [IgG], and nonstructural protein 1 [NS1] antigen tests) in 60 clinically suspected patients (April 2013 to August 2015). NS1 protein was detected in 5% ($N = 3$), at least one of the antibodies (IgM and/ or IgG) was detected in 11% ($N = 7$) of the samples. Five patients identified as of acutely infected. There was a simultaneous presence of NS1 protein and IgG or IgM antibodies in 4% ($N = 2$) of patients. Previous studies show establishment of potential vectors in this area. These evidences support the hypothesis that DF can be a health concern in Southeast Iran with potential future outbreaks.

INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are two of the most widely spread mosquito-borne disease in Southeast Asia, western Pacific region, and the United States. The disease is caused by dengue virus (DENV), a Flaviviridae with four closely related serotypes (DEN-1, DEN-2, DEN-3, and DEN-4).[1] It is estimated that over 3.9 billion people or nearly half of the world's population are susceptible to this virus and about 50 million people are infected with DENV annually. DF presents with a wide range of clinical manifestations from subclinical symptoms or mild fever to severe form of DHF or dengue shock syndrome.[1]

Iran has always been prone to DENV mainly due to its geographical location and bordering with DENV endemic countries such as Afghanistan and Pakistan. In 2011, more than 17,000 DF cases were diagnosed in Pakistan with 219 deaths.[2] In 2013, more than 74,000 cases were confirmed in India.[3] In addition, there are some sporadic cases of DF reported in multiple provinces of Afghanistan.[4]

Dengue fever has started to be a concern in Iran since 2008, after the first case was confirmed in a 61-year-old man with a travel history to Malaysia.[5] After this, in a retrospective study on 300 previously collected samples (2000–2012), 15 (5%) and 3 (1%) of the specimens were positive by serology and serology plus polymerase chain reaction (PCR) for DF, respectively. Of the 15 seropositive cases, 7 (46.7%) cases did not have any travel history outside Iran; six cases were collected from the residents in Sistan and Baluchestan province.[6] As a result, this area was a focus of more investigation to understand burden of this disease. This area is adjacent to Pakistan and has hot-dry climate, which could potentially increase the chance of emergence of *Aedes* mosquitos. One of the most possible scenarios in this region is the chance of misdiagnosing DF with Crimean Congo hemorrhagic fever (CCHF). CCHF is currently endemic and known in this area. Therefore, the present study was designed to investigate possibility of DF in patients clinically suspected of having viral hemorrhagic fever but tested negative for CCHF in Sistan and Baluchestan province of Iran.

METHODS

Sample collection. The study protocol was approved by Institutional Ethics Committee (Approval No. IR.ZAUMS.REC.1393.7002) at Zahedan University of Medical Sciences. Serum specimens were collected from suspected patients admitted to Boo-Ali Hospital in Zahedan within the first 3 days of admission (April 2013 to August 2015). Suspected cases were interviewed and examined by infectious diseases physicians. Patients presenting with compatible symptoms (fever, myalgia, arthralgia, headache, rash, or bleeding) and tested negative for CCHF (PCR, immunoglobulin M [IgM], and immunoglobulin G [IgG]) were recruited to the study. In addition, seven samples were also sent to the hospital from the rural area of Baluchestan district (Saravan) from suspected patients presented with similar presentations within the first 3 days of symptoms onset. Samples were tested for anti-dengue virus IgM, IgG, and nonstructural protein 1 (NS1) antigen.

Test performance. Virus isolation, PCR, or antigen detection can be used to diagnose DF during acute febrile illness. Unfortunately, PCR and viral culture were not available at the time of study, and we decided to use combination of serology and antigen detection. IgM, IgG antibodies, and NS1 antigen were tested using commercial enzyme-linked immunosorbent assay (ELISA) kits provided from Euroimmune AG, Luebeck, Germany (reference no: EI 266b-9601 M, EI 266b-9601 G, and EQ 266a-9601-1, respectively). The optical density (OD) of each sample was examined at the wavelength of 450 nm and the reference wavelength was 620–650 nm. The OD of samples was compared with the calibrator. Per manufacturer's instruction, the result was interpreted negative if the ratio of the sample reading to caliber was <0.8, borderline if the ratio was ≥ 0.8 and <1.1, and positive if the ratio was ≥ 1.1.

RESULTS

In this study, a total of 60 patients (36 males and 24 females) met inclusion criteria. Overall, 13 patients (7 males and 6 females; mean age of 30 years) had evidence of past or recent exposure to DENV (Table 1). Five patients had positive test

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TABLE 1

Result of dengue virus test studies in 13 Iranian patients presenting with fever, rash, headache, and myalgia in Sistan and Baluchestan, Iran (2013–2015)

Cases	Sex (male/female)	Age (years)	Occupation	Locality (City)	Anti-dengue IgG (ELISA)	Anti-dengue IgM (ELISA)	Dengue NS1 Antigen (ELISA)	Interpretative test result
Case 1	Male	58	Farmer	Zahedan	Borderline	Negative	Negative	*Possible previous exposure*
Case 2	Male	30	Worker	Zahedan	Negative	Borderline	Negative	*Possible acute infection*
Case 3	Female	5	NA	Saravan	Negative	Borderline	Negative	*Possible acute infection*
Case 4	Female	8	NA	Saravan	Negative	Positive	Negative	Acute infection
Case 5	Male	24	Worker	Saravan	Positive	Negative	Positive	Previous exposure with possible acute infection
Case 6	Female	37	Housewife	Saravan	Negative	Negative	Positive	Acute infection
Case 7	Female	47	Housewife	Saravan	Negative	Negative	Borderline	Not sure
Case 8	Male	47	Worker	Saravan	Negative	Positive	Positive	Acute infection
Case 9	Female	30	Housewife	Saravan	Negative	Borderline	Negative	*Possible acute infection*
Case 10	Male	18	Student	Zahedan	Positive	Borderline	Negative	Previous exposure with possible acute infection
Case 11	Male	35	Worker	Zahedan	Positive	Borderline	Negative	Previous exposure with possible acute infection
Case 12	Female	23	Housewife	Zahedan	Negative	Positive	Negative	Acute infection
Case 13	Male	27	Self-used	Zahedan	Negative	Positive	Negative	Acute infection

NA = not applicable.

results in favor of acute infection. None of patients had travel history outside Iran.

IgM antibodies were detected in 6.5% (4) patients. Five samples were reported as borderline. Unfortunately, we could not repeat blood draw to confirm diagnosis in these five cases due to loss of follow-up. IgM and NS1 protein were both positive in one case.

IgG antibodies were detected in 5% (3) patients. One patient had a borderline report. IgG and NS1 antigen were positive in one case without detectable IgM antibodies. None of patients with positive IgG had positive IgM against DENV, but two had borderline IgM detected.

NS1 antigen was detected in 5% (3) patients. All positive results were rechecked and confirmed. One patient had a borderline NS1 antigen report with negative IgG and IgM. Two patients with positive NS1 antigen had either IgM or IgG antibodies.

Leukopenia and thrombocytopenia were the most common laboratory manifestations. All patients received supportive care (fluid resuscitation, electrolyte replacement, and transfusions if required). No antiviral therapy was administered. No patient required intensive care. All patients survived and discharged from hospital.

DISCUSSION

The DENV is one of common public health problems in particular areas where both the virus and its vector overlap with human settlement which may bring a high risk of outbreaks. Several natural variables such as temperature, rainfalls, structural changes in virus, and vector population can cause the spread of DENV in new environments.[7,8] Other factors such as vector transportation through commercial shipments and trespassing of human population can dramatically increase the risk of virus transportation to the areas where suitable vector is available.[9,10]

Iran is one of countries with high risk of DENV emergence due to its critical geographic location. Southeast Iran is in proximity to DF endemic countries such as Pakistan.[2] As similar studies pointed out, increased trading activities and trespassing across Iran's southeast boarders may significantly increase emergence of DENV or efficient vectors in this area.[11] Recent studies in Pakistan show a high rate of DENV circulation in different areas (Punjab, Sindh, and Baluchestan).[12,13] In a previous study conducted in 2012, 15 of 300 previously collected specimens in Iran's Pasteur Institute were seropositive against DENV with six positive samples from the Sistan and Baluchestan province.[6] Our study was designed in follow up of this previous report and could detect at least five patients with acute DENV infection in this area.

The possibility of cross reactivity between dengue serology tests and other flaviviruses (West Nile virus [WNV], St. Louis encephalitis virus, Japanese encephalitis virus and yellow fever virus [YFV]) has been considered. None of patients had recent or previous encephalitis symptoms. Although WNV cases and positive serologies have been reported in north and central Iran,[14–16] there is no report of WNV from the southern part. YFV has not been reported from Iran, and none of patients were vaccinated against YFV. Moreover, we do not expect cross reactivity with DENV NS1 antigen. Overall, all evidences support validity of our test results.

A recent study (2008–2014) has identified *Aedes albopictus* in southern Iran.[17] The species *A. albopictus* is most well-known for transmitting dengue and chikungunya viruses. In another study, *Aedes unilineatus* was also identified in the southeast of Iran (2012–2014). This mosquito species has been reported as a dengue vector in Karachi, Pakistan.[18] These studies support establishment of DENV vectors in this area.

This study supports the hypothesis that DENV circulates in patient population in the southeast of Iran and reflects the fact that the risk of DENV outbreaks in this area is greater than what was imagined before. These results could be also evidence of small outbreaks which were not large enough to attract attention from public health authorities, although establishing a national surveillance system to monitor annual number of cases throughout the country would be an ideal response to this report to collect data and establish infrastructures for future research work and outbreak response. Finally, studies for finding other potential vector species, that is, *Aedes aegypti* mosquitos in this area can provide researchers with useful insights on DENV and its epidemiology in Iran.

The study has limitations. Serology tests are not confirmatory in any type of infections, but can be used as an alternative mode of diagnosis. We would highly recommend using confirmatory tests (PCR and viral culture) in future studies. Moreover, anti-dengue IgM antibody is expected to be usually reactive by day 5, as a result number of DF cases detected in this study might have been underestimated due to early testing.

Received August 10, 2017. Accepted for publication October 9, 2017.

Published online March 19, 2018.

Financial support: This study has been funded by Zahedan University of Medical Sciences.

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