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Introduction of Zika virus in Bangladesh: An impending public health threat

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ABSTRACT

Objective: To explore the presence of Zika virus (ZIKV) in Bangladesh and to understand the associated risk factors.
Methods: A retrospective sero-surveillance was performed on stored serum samples of dengue surveillance conducted from 2013 to 2016. Real time RT-PCR was performed on randomly selected acute serum samples to detect the Zika virus nucleic acid.
Results: Of 200 samples screened, one was found positive for ZIKV by real time RT-PCR and further confirmed by genome sequencing. The case was a 65 years old male from a metropolitan city of Bangladesh who had no history of travel outside Bangladesh. Phylogenetic analysis of partial E gene sequences from Bangladeshi isolates demonstrated a close relationship with ZIKV from Brazil and current South American strains clustering within a monophyletic clade distinct from African lineage.
Conclusions: Presence of ZIKV raises serious public health concerns in Bangladesh owing to its association with congenital anomalies/neurological-manifestations. We, therefore, recommend every suspected viral fever patient, particularly pregnant women be screened for ZIKV infection to rule out yet another emerging infection in Bangladesh.

1. Introduction

Zika virus (ZIKV) – an arthropod-borne virus which belongs to family Flaviviridae [1] was first isolated in Uganda in 1947 from Rhesus monkeys [2]. It was then emerged in Africa as sporadic benign-human infections during the early 1960s through the middle of 1980s. Later, in 1969, ZIKV appeared as a potential pathogen in Asian-continent. The first ZIKV was isolated from Aedes mosquitoes in Malaysia [3] and human cases were confirmed in Indonesia and Pakistan in the early 1980s [4–6]. Several studies also documented widespread population exposure with ZIKV [5,6] but with mild clinical manifestations and since no outbreak had been reported, therefore, ZIKV failed to attract enough attention, globally.

Concerns started growing since 2007 when ZIKV-epidemics broke out in Yap Island, Micronesia and another large outbreak in French Polynesia during 2013–2014 [7]. More severe public health implications started revealing since early 2015, when a huge ZIKV outbreak swept over Brazil being associated with microcephaly &/or other neurological disorders [8]. In March 2017, WHO confirmed active ZIKV infections in 61 countries, the majority of which are from America, Africa and Western Pacific regions. In South and South-East Asia except Singapore, ZIKV cases are remain unexpectedly low comparing to America and Caribbean countries [9]. Nevertheless, epidemiology, geographical location and factors like distribution of Aedes-mosquito, effect of global warming on climate change in vector density [10] and trans-continental traveling suggest that there should be more ZIKV-cases in Asian regions since previous serological evidence attested this assumption clearly.

In Bangladesh, some epidemiological, clinical and outbreak studies were conducted on dengue [11] and chikungunya [12] in contrast to ZIKV, which might has been missed or ignored due to lack of its attention earlier than 2015. These aforementioned facts and figures and serious public health implications of ZIKV infection pertaining to microcephaly/neurological manifestations prompted us at the Institute of Epidemiology, Disease control and Research (IEDCR) to explore if any ZIKV could be detected

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using molecular method (no serological test was then available) from the serum collected from febrile patients of on-going dengue surveillance conducted in three selected districts of Bangladesh during 2013–2016.

2. Materials and methods

2.1. Study population and sample collection

From programmed archived data of 2013–2016, 576 patients were selected systematically who fulfilled the inclusion criteria of continuous fever for ≤5 d and any two of the clinical features like headache, myalgia, arthralgia, leucopenia, thrombocytopenia, body rash and non-purulent conjunctivitis. Due to resource restraints, only 200 (50 from each of 4 years) serum samples were randomly selected.

2.2. Detection of Zika virus using real time RT-PCR

From all the 200 serums RNA were extracted using RNeasy Mini Kit (Qiagen, Crawley,UK) and were tested for presence of Zika virus RNA using RealStar® RT-PCR Zika virus Kit (Altona Diagnostic, CA, USA) following manufacturer's instruction where 5 μL mastermix A, 15 μL mastermix B and 1 μL internal control were used for PCR reaction with the thermal profile as 55 °C for 20 min 1 cycle, 95 °C for 2 min 1 cycle and 95 °C for 15 s followed by 55 °C for 45 s 45 cycles in a ABI 7500 Fast Dx machine (Applied Bio system, USA).

2.3. Nucleotide sequencing

Positive sample as detected by the real time PCR was then subjected to polymerase chain reaction (PCR) for sequencing of partial E gene using the forward primer GTA CTT GGA TAG GAG CGA TGC and reverse primer TTG CTT GTC AAG GTA GGC TTC. The amplified product (723 bp) from PCR was then purified using Purelink PCR Purification kit (Thermo Fisher Scientific Inc., Massachusetts, USA). Cycle sequencing of purified PCR product was performed using Big Dye Version 3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). Then cycle sequencing product was again purified using BigDye®X Terminator™ purification kit (Applied Biosystems) as per manufacturer's protocol, as well. Finally purified cycle sequencing product was subjected to capillary sequencing in an ABI PRISM 310 Automated Sequencer (Applied Biosystems).

2.4. Sequence analysis

The chromatogram sequencing files were inspected using Chromas 2.23 (Technelysium, Queensland, Australia), and the consensus sequence was prepared using SeqMan II (DNASTAR, Madison, WI). Reference sequences were retrieved from GenBank and analyzed using Bio-Edit sequence alignment editor, v. 7.0.1.

2.5. Phylogenetic analysis

A consensus sequence of around 630 bp was prepared using both forward and reverse primers generated sequencing data, which was used for analysis of ZIKV lineage. Phylogenetic analysis was conducted using MEGA version 6 (Tamura, Stecher, Peterson, Filipski, and Kumar 2013). The dendrogram was constructed by using the neighbor-joining method.

2.6. Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Review Board of IEDCR for secondary analysis of preserved samples collected for the study "Impact of Climate Change on Dengue". All patients were informed on the purpose and intent of the study and procedures involved. Written informed consent from all participants (and assent from minor's parents) was taken. Participation in the study was voluntary. A semi structured questionnaire was used to document personal details and other relevant medical data by the physicians of sentinel surveillance sites. In the main database, every patient was coded with a unique identifier (anonymous, numerical) and their blood samples were sent to the laboratory and the test results were recorded by de-coded the ID (s) by the PI and entered into main database.

3. Results

3.1. Findings of real time RT-PCR analysis

Among 200 samples tested, one was found positive for ZIKV infection. Positive sample was further subjected to sequencing to confirm the real time-PCR data, sequencing of 723 bp target and alignment with reference sequence confirmed ZIKV infection. The nucleotide sequence data has been submitted to GenBank, NCBI under accession number KY064008.

3.2. Findings of phylogenetic analysis

A dendrogram was constructed using sequencing data (630 bp contig) obtained from study sample and reference strains from different geographical regions and period/times available in GenBank database (Figure 1). The Bangladeshi strain clustered together with the currently circulating strains belonging to Asian lineage.

3.3. Outbreak investigation and clinico-epidemiological findings

After the laboratory confirmation, our pre-trained outbreak investigation teams from IEDCR immediately initiated an epidemiological investigation on the case and surrounding localities. Archived data showed that the patient reported at the dengue surveillance site of Chittagong Medical College Hospital where the blood sample was collected in August 2014. The patient was a 65 years-old male presented with fever for five days with maculopapular rash and body ache. No other sign symptom was recorded. He had no history of traveling outside the country within the last 2 weeks of onset of fever/other relevant signs/symptoms.

During outbreak investigation in March 2016, the patient was found in sound health neither having any complain of focal &/or generalized neurological deficit. The investigation team also conducted a quick survey in patient's household and in

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