Molecular surveillance of dengue infections in Sabya governate of Jazan region, Southwestern Saudi Arabia

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Abstract

Dengue fever is considered to be the most important mosquito-borne disease and considered as endemic disease in Jazan region, Saudi Arabia. The present study was aimed to analyze the prevailing dengue virus serotypes in Sabya governate of Jazan region. 100 Serum samples of NS1 positive cases samples were collected from November 2015 to October 2016 and tested by one step Reverse Transcription Polymerase Chain Reaction (RT-PCR) with a set of specific primers for detection of four dengue virus serotypes followed by sequencing the PCR products to confirm the results. Out of the 100 serum samples, 33 were found positive for dengue infection (33.0%). Two dengue virus serotypes were detected; DEN-2 and DEN-3, DEN-2 is the most common and predominant type in tested samples 69.7% (23/33) and then DEN-3 30.3% (10/33). The high seroprevalence of dengue virus infections in Sabya governate indicates its endemicity. The present study highlights the importance of tracking the spread of dengue virus types and its implication for analyzing changes in dengue endemicity in specified areas over time. Complete genome sequencing is required for the two detected dengue virus in the governate (DEN-2 and DEN-3) to serve as references for any future epidemiological researches and/or outbreaks.

Keywords: dengue fever, serotypes {2 & 3}, Sabya-Jazan region, Saudi Arabia

1. Introduction

In brief the fact sheet of World Health Organization (WHO, updated July 2016) reported that the Dengue virus disease has grown dramatically, resulting in that half of the world’s population living in tropical and subtropical regions now at risk, now the disease is endemic in more than 100 countries comparing with 9 countries reported the disease before 1970. The first isolation of dengue virus was in the second world war from American servicemen stationed in southeast Asia and western pacific Ocean islands (Gubler. 1998) [11]. The virus belong to flaviviridae family and genus flavivirus, dengue virus is a positive – sense, single strand RNA virus of 10700 nucleotides which encodes three structural proteins, C (core protein), M (membrane protein) and E (envelope protein) and seven non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 composed of four serotypes known as DENV1, DENV2, DENV3 and DENV4. (2, 3 and 4). Dengue virus is one of the most important arboviral disease of humans (mosquito-borne disease), meaning that the transmission from person to person and to complete its life cycle requires bite (blood-sucking) of infected female mosquito. DENV is able to replicate in human and mosquito cell (tow hosts human and mosquitoes) (Lindenbach, 2003) [16]. The main and most efficient DENV vector is Aedes aegypti and Aedes albopictus (Gubler. 2002) [12]. First outbreak of dengue fever in Saudi Arabia reported in Jeddah 1994, 289 confirmed cases of the viral infection, since this year dengue fever occurring annually in Jeddah. Western and Southwestern of the country considered to be endemic areas after many outbreaks occurred in this areas (Jeddah, Makka, Jazan and Aseer) and all the previous studies in the Saudi Arabia (Jeddah and Makka) confirmed that the circulating dengue virus serotypes were DENV 1, DENV 2 and DENV 3. (Fakeeh and Zaki 2003: Khan et al., 2008: Al-Azraqi et al., 2013) [9, 13, 3].

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2. Materials and Methods

Study area
Jazan Region in Southwest Saudi Arabia lies between 16°-12, and 18°-25, latitude north. It is bordered in the South by Arabic republic of Yemen with total area of about 22,000 km² and 1.3 million populations (Census 2011). Thirty percent of the population concentrated in six major cities, and the remainders living in over 3500 villages (Alsheikh, 2011) [1]. Jazan region is situated in the subtropical zone and has average monthly temperatures ranging between 25.8°C in January to 33.4°C in July. The average relative humidity ranges between 55% and 72.5%. The rainy season is started at August through October with a monthly average of 77 and 56.7mm, respectively (Alsheikh, 2011) [1]. Jazan is divided into eleven small Governates (Al-Aridah, Damad, Twal, Al-Ahad, Jazan, Al-Khobah, Samitah, Abuareesh, Sabya, Beash and Al-Darb), these locations although with different altitudes and geographical Characteristics, they are almost share the same demographical, agricultural, educational, cultural, housing, health system, and environmental characteristics.

Sampling
From November 2015 to October 2016 about 390 suspected dengue fever patients serum samples included in this study were collected from Sabya governate in Jazan region and stored at -80° till further use.

Table 1: Oligonucleotide primers used in RT-PCR and Nested-PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5 – 3</th>
<th>Genome position</th>
<th>Size in bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>TCAATATGCTGAAACGCGCAGAAACCG</td>
<td>134-161</td>
<td>511</td>
</tr>
<tr>
<td>D2</td>
<td>TGTGACAAACAGTCATGCTTCTCAGGTFCT</td>
<td>616-644</td>
<td>511</td>
</tr>
<tr>
<td>TS1</td>
<td>CGTCCTCAGTGATCCCGGGG</td>
<td>568-586</td>
<td>482 (Dl and TS1)</td>
</tr>
<tr>
<td>TS2</td>
<td>CGGCCAAGGGCCCATGAAACAG</td>
<td>232-252</td>
<td>119 (Dl and TS2)</td>
</tr>
<tr>
<td>TS3</td>
<td>TAACATCATCAGGACAGACC</td>
<td>400-421</td>
<td>290 (Dl and TS3)</td>
</tr>
<tr>
<td>TS4</td>
<td>CTCTGTGTCTCTAAACAGAGA</td>
<td>506-527</td>
<td>392 (Dl and TS4)</td>
</tr>
</tbody>
</table>

The samples were subjected to initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation (94°C, 30 s), primer annealing (55°C, 1 min), primer extension (72°C, 2 min) and final extension for 5 minutes. In each run negative and positive controls were included. The PCR products of nested amplification were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium promide. The visualization was carried out using Gel Doc XR Imaging System (Bio-Rad).

One hundred samples out of 186 (56.4%) NS1 positive cases samples tested by RT-PCR.

RNA isolation
Reall.ine Nucleic Acid Extraction Kit from Bioron diagnostic (Germany) used for extraction of RNA according to the manufacturer’s instructions.

Reverse transcriptase polymerase chain reaction (RT-PCR)
One step RT- PCR is a rapid, sensitive, and simple for dengue serotype specific diagnosis method. The test was performed according to the protocol of Lanciotti et al. (1992) [15] with some modification; DEN consensus primers and serotype-specific primers (Table 1) were used to amplify the viral genome in this study and synthesized in Integrated DNA Technology (Belgium). The one step RT-PCR reactions were performed according to access RT-PCR–system protocol (Promega-USA) in total volume of 50 μl containing 10 μl of AMV/Tfl 5X Reaction Buffer, 1 μl of dNTP Mix (10mM each dNTP, final concentration 0.2mM), 2 μl of 25mM MgSO4 (final concentration 1mM), 1 μl of AMV Reverse Transcriptase 5u/μl (final concentration 0.1u/μl), 1 μl of Tfl DNA Polymerase 5u/μl (final concentration 0.1u/μl), 50pmol (final concentration 1μM) of each forward (D1) and reverse (D2) primers, 5 μl of RNA virus and nuclease free water to total volume 50 μl. The thermal cycling incubations temperatures programmed as follows: incubation for 1 hour at 42°C (to convert the RNA to cDNA) then initial denaturation for 3 minutes at 94°C followed by 35 cycle of denaturation (94°C, for 30 second), primers annealing (55°C for 1 minute), primer extension (72°C for 2 minutes) and final extension for 5 minutes.

Nested-PCR
Nestet PCR was performed in 2 tubes for each sample in 50 μl reaction mixture containing 25 μl GoTag®G2 green master mix ready to use from Promega, 10 μl of the diluted (1:100) RT-PCR product, 50 pmol (final concentration 1μM) of each forward primer D1 and TS1, TS3 as reverse primers for the first tube and TS2, TS4 as reversers primers for another tube.

Sequencing and bioinformatics analysis
Purification and standard sequencing for RT- PCR products were performed by Macrogen Company (Seoul, Korea). Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each
template using D1 (forward) primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with Big Dye®X Terminator™ purification protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). The sequences were searched for sequence similarity through BLAST (www.ncbi.nlm.nih.gov/BLAST/) (Atschul et al., 1997) [4] and compared to reference sequences of Dengue serotypes detected in BLAST and downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/).

Similarity tree was obtained from database online by phylogeny.fr (http://www.phylogeny.fr/).

3. Results

RT-PCR and Nested-PCR

One hundred samples out of 186 (56.4%) NS1 positive cases samples tested by RT-PCR. Thirty-three out of 100(33.0%) were confirmed positive for dengue virus when using D1 and D2 primers (511bp) for all serotypes, and the RT-PCR product was used as a sample for the nested-PCR using a set of serotype-specific primers pair as described in the methodology. Two dengue virus types (DEN-2 and DEN-3) were detected and the results showed that DEN-2 is the most common and predominant type in Sabya governate of Jazan region rating twenty-three out of thirty-three (69.7%), followed by DEN-3 ten out of thirty-three (30.3%), and serotype 1 & 4 was not detected (Figure1: Table 2).

Sequencing

To confirm the serotype-specific results, the partial sequencing was done for nineteen RT-PCR product samples represent the three serotypes (DEN-2 and DEN-3). The Blast search showed that the sequences of our samples aligned along with many published sequences of dengue virus serotypes as shown in (Table 3 and Fig.4 and Fig.5) and similarity tree (Fig.6 and Fig.7) which illustrates the Gen bank accession numbers and the country of isolates.

Sequencing of DEN-2 in this study revealed that it is in close similarity to varies Indian types (Table 3, Fig.4, and Fig.6), while DEN-3 is in similarity to some Asian types including India, China, and Singapore (Table 3, Fig.5, and Fig.7).

Table 2: Results of RT-PCR and nested-PCR

<table>
<thead>
<tr>
<th>No of tested samples</th>
<th>^Ve DEN</th>
<th>^Ve DEN-1</th>
<th>^Ve DEN-2</th>
<th>^Ve DEN-3</th>
<th>^Ve DEN-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>33 (33%)</td>
<td>0 (0%)</td>
<td>23 (69.7%)</td>
<td>10 (30.3%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 3: Results of Blast search

<table>
<thead>
<tr>
<th>DEN-2</th>
<th>DEN-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gen bank accession No</td>
<td>Country</td>
</tr>
<tr>
<td>JN935383</td>
<td>India</td>
</tr>
<tr>
<td>KU351296</td>
<td>India</td>
</tr>
<tr>
<td>GU968539</td>
<td>India</td>
</tr>
<tr>
<td>KX577706</td>
<td>China</td>
</tr>
<tr>
<td>KT180256</td>
<td>India</td>
</tr>
</tbody>
</table>

Fig 1: (A) Agarose gel electrophoresis of RT-PCR using D1-D2 primers. Lane 1: 100bp ladder, lane 2: positive control, lane 3, 5, 6 and 7 negative samples, land 4 and 8 positive samples.
(B) Agarose gel electrophoresis of nested PCR using D1-TS2 primers. Lane 1: 100bp ladder, lane 2: positive control, lane 3, 6 and 7 positive samples, land 4, 5 and 8 negative samples.
(C) Agarose gel electrophoresis of nested-PCR using D1-TS32 primers. Lane 1: 100bp ladder, lane 2: negative control, lane 3: positive control, lane 4, 6, 7and 8 negative samples, land 5 positive samples.
Fig 4: Identities between DEN-2 from Sabya and DEN-2 of India (gb|JN935383.1) Dengue virus strain VCRC/DENV2/03/10 polyprotein gene, partial cds Length=508, Score = 848 bits (459), Expect = 0.0, Identities = 471/476 (99%), Gaps = 3/476 (1%), Strand=Plus/Plus

Fig 5: Identities between DEN-3 from Sabya and DEN-3 of India (gb|KF954949.1) Dengue virus 3 isolates 13GDZDVS30E, complete genomeLength=10677 Score = 861 bits (466), Expect = 0.0, Identities = 473/476 (99%), Gaps = 1/476 (0%), Strand=Plus/Plus
Dengue virus isolate TAN531 polyprotein gene, partial cds
  Dengue virus 2 isolate 17/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 20/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 21/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 26/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 30/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 27/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 29/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 22/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 23/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus isolate 4/D2/Del/2013 polyprotein gene, partial cds
  Dengue virus 2 isolate 1/D2/Del/2012 capsid/premembrane protein gene, partial cds
    Dengue virus isolate 7/D2/Del/2014 polyprotein gene, partial cds
    Dengue virus isolate 6/D2/Del/2014 polyprotein gene, partial cds
    Dengue virus isolate 7/D2/Del/2013 polyprotein gene, partial cds
    Dengue virus 2 isolate 34/D2/Del/2013 polyprotein gene, partial cds
    Dengue virus 2 isolate 32/D2/Del/2013 polyprotein gene, partial cds
      Dengue virus isolate RMRC-VRDL-01060/2015 polyprotein gene, partial cds
        Dengue virus 2 isolate 33/D2/Del/2013 polyprotein gene, partial cds
        Dengue virus isolate 2/D2/Del/2014 polyprotein gene, partial cds
        Dengue virus isolate 1/D2/Del/2014 polyprotein gene, partial cds
        Dengue virus strain VCRC/DENV2/02/10 polyprotein gene, partial cds
          Dengue virus isolate RMRC-VRDL-01078/2015 polyprotein gene, partial cds
            Dengue virus 2 isolate 2/D2/Del/2012 capsid/premembrane protein gene, partial cds
              Dengue virus 2 isolate 6/D2/Del/2012 capsid/premembrane protein gene, partial cds
              Dengue virus 2 isolate 28/D2/Del/2013 polyprotein gene, partial cds
              Dengue virus isolate Den-M11/2012 CprM gene, partial cds
              Dengue virus 2 isolate 18/D2/Del/2013 polyprotein gene, partial cds
              Dengue virus isolate 10/D2/Del/2013 polyprotein gene, partial cds
                viruses | 6 leaves
                Dengue virus 2 isolate Den-M23/2012 polyprotein gene, partial cds
                  viruses | 57 leaves
                  Dengue virus 2 isolate 8/D2/Del/2012 capsid/premembrane protein gene, partial cds
                    viruses and unknown | 2 leaves

Fig 6: DEN-2 serotype similarity tree.
4. Discussion
Fast means of travel, country interdependencies, mass migration from rural to urban and from endemic to non-endemic countries or vice versa have increased the opportunities for contact between people of different nationalities, races and cultures. Some of the above factors are compounded in the unique situation presented in Saudi Arabia which is a vast subtropical country situated in the center of the Islamic World, with unique movement of population from all over the world and in-between these cities resulting in a unique epidemiological significance (Khan et al., 2008) \(^{[13]}\). The interest in vector-borne diseases has recently increased.
worldwide. Infection with DENV produces a wide spectrum of clinical features ranging from asymptomatic or non-specific influenza-like undifferentiated fever in more than 50% of infected individuals, or viral symptoms typical DF to a severe and fatal dengue haemorrhagic fever/dengue shock syndrome (Gubler 1998) [11]. Thus, diagnosis of DENV infection on the basis of clinical symptoms is not reliable, and the diagnosis should be confirmed by laboratory tests with rapid detection and serotyping of dengue viruses. Diagnosis of DENV infection based on commercially available ELISA-based serological assay, a relatively simple test. However, the assay has many limitations and drawbacks. It cannot determine DENV serotype. It also detects cross reacting antibodies to other pathological conditions leading to apparently false positive results at high rates of up to 42.5% (Wilder-Smith and Schwartz 2005). These pathological conditions include various flaviviruses such as Japanese encephalitis (JE) virus and yellow fever (YF) virus, tick-borne encephalitis virus, St. Louis encephalitis virus and/or West Nile virus, in addition to the presence of rheumatoid factor in patients with autoimmune diseases (Chana et al. 2004) [8].

Cases of JE and YF do not exist in Sabaya or Saudi Arabia as a whole, and no subjects with previous JE or YF immunizations were included in the study. However, many Saudis are travelling to endemic countries for business or vacations, where they are at risk of catching vector-borne diseases and being misdiagnosed and going unnoticed or unreported. Therefore, false positive results, because of cross-reactivity, are still a potential issue. We did not conduct a population-based randomized study as we were reporting just prevalence, and therefore, a recruitment bias is possible; however, this bias is likely to be small as Sabaya large hospitals attract visitors and staff from different areas of Jazan region.

No previous data on the dengue prevalence situation in Sabaya are available, but a similar study conducted in Jazan, Saudi Arabia reported prevalence of three serotypes (Den 1, Den 2 and Den 3) in One hundred twenty-four positive samples out of 220 (56.4%) (Alsheikh et al., 2017) [2].

The diagnostics of imported viral infections such as DF is often performed with commercial tests not subjected to regular quality control regimens and clearly demonstrated differences in sensitivity and specificity. Patients with positive IgM 4–8 days from the onset of fever and negative for PCR were most likely considered by studies to be misclassified as having acute dengue infection (Kuno 1998) [14].

The pattern of distribution of DENV serotypes detected in this study population showed that DENV-2 was the most predominant dengue virus type, a result which is in line with the reports of Fakeeh and Zaki (2001, 2003) [8, 9] and Zaki et al. (2008) who stated that DENV-2 virus is the predominant serotype in Saudi Arabia particularly in western Saudi Arabia since 1992. El-Kafrawy et al. (2016) [7] showed that DENV-2 isolate from Jeddah belongs to the Cosmopolitan genotype was most genetically related to isolates from Pakistan circulating from 2008 to 2013. The three dengue virus serotypes DEN-1, DEN-2, and DEN-3 are thought to be predominant in the Middle East, especially in Yemen and Saudi Arabia (Nedjadi et al., 2015) [17].

Nucleotide sequence of 240-bp E/NS1 junctions of 81 dengue viruses was isolated from cases in Jeddah, Saudi Arabia from 1994 to 2006 (Zaki et al. 2008) [20]. Three serotypes (DENV-1, DENV-2 and DENV-3) were circulating, with more than one serotype in each outbreak. DENV-1 and DENV-2 were recorded in 1994 outbreak, while DENV-3 emerged in 1997. In 2004, all three serotypes were isolated, and DENV-1 was isolated from the summer of 2005 to early 2006 (Zaki et al. 2008) [20].

Results indicated that dengue fever prevalence in Sabaya (33%) compared to the previous reports of Alsheikh et al. (2017) [2] Al-Arzaqi et al. (2013) and Gmael et al. (2014) [10] who reported dengue prevalence of 56.4, 26.5% and 47.74%, respectively, in the Jazan region. In this study, two dengue virus types (DEN-2 and DEN-3) were found circulating in Sabaya governate of Jazan region with the predominance of DENV-2 scoring 23 out of 33 dengue positive RT-PCR samples (69.7%), followed by DEN-3 (10 out of 33 – 30.3%), however serotype 1 and 4 was not detected in this study.

The similarity of DEN-2 to varies Indian types, in addition to, the similarity of DEN-3 to some Asian types including India, China, and Singapore suggested the likelihood of introduction of these serotypes to Sabaya governate either by travelling from and to those countries especially the migrant labors (DEN-1, DEN-2, DEN3.), or through direct introduction from Jeddah and Yemen.

5. Conclusion

In conclusion, data obtained from this study are of value in increasing the awareness for practicing doctors and other healthcare personnel to consider DENV fever as a part of their differential diagnosis when confronted with febrile illnesses and commence relevant case management.

Intensive social interventions are required to increase community awareness of dengue to minimize the risk of the severe complications of this infection. Further detailed studies and operational research with more advanced epidemiological methodologies are important to fill the existing knowledge gaps in terms of dengue dynamics in the region.

6. References


