Epidemiological screening and serotyping analysis of dengue fever in the Southwestern region of Saudi Arabia

Alkhansa Alshabi, Amani Marwan, Nuzhath Fatima, Aymen M. Madkhal, Fatemah Alnagai, Abrar Alhazmi, Hesham M. Al-Mekhla, Ahmed A. Abdulhaq, Khalid Y. Ghailan, Ahmed Salid, Tareq Refaei

A Department of Medical Laboratory Technology, College of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia
B Medical Research Center, Jazan University, Jazan, Saudi Arabia
C Department of Epidemiology Public Health and Tropical Medicine College, Jazan University, Jazan, Saudi Arabia
D Public Health Office, Jazan, Saudi Arabia
E Department of Laboratory, King Fahd Hospital, Jazan, Saudi Arabia

Abstract

Dengue is an acute systemic viral disease that has been developed globally in both chronic and epidemic transmission periods. Dengue virus (DENV) is a member of the Flavivirus genus of the Flaviviridae family, which endangers public health. Limited studies have been performed in the Saudi Arabia and there are no epidemiological as well as molecular screening of DENV in the Southwestern region and this current study was conducted on the epidemiology of dengue in the Southwestern region of Saudi Arabia. Simultaneously, we have screened the 100 patients for DENV using the real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay. The current study results confirmed that 6637 people from various hospitals and locations in Jazan, Southwestern regions, were enrolled in this study from 2012 to 2020. The overall mean age was 30.02 ± 18.01 years, with 62.8% of males and 37.2% of females enrolled. This study included nearly three-fourths of the Saudi participants and one-fourth of the expatriates, and 56.6% of the positive cases were enrolled. In 2019, the most instances were enrolled, with 44% of positive cases. When screened using the RT-PCR assay, 93% of the positive patients were recruited, according to the quality control analysis. In conclusion, the current study results confirmed the prevalence of DENV was increased drastically since 2012 to 2020. High number of cases were registered prior to the Pandemic. The screening for DENV was performed with RT-PCR assay and NSI antigen should also be implemented to cross-check the results which was previously performed with RT-PCR analysis.

1. Introduction

Dengue Fever is a mosquito-borne viral infection causing serious flu-like diseases that often lead to a life-threatening condition known as dengue shock syndrome (DSS) (Liu et al., 2021). Inapparent Flavivirus (Flaviviridae) genus dengue virus (DENV) infections lead to a wide range of clinical involvement ranging from mild febrile disease to severe DSS (Dengue Hem) (Anoopkumar and Aneesh, 2021). Dengue in humans is transmitted by Aedes aegypti and Aedes albopictus mosquitoes (Weiskopf and Sette, 2014). Extreme epidemics of dengue in nine countries before 1970 alone, but currently, in tropical and subtropical areas, dengue fever has had an effect on more than 100 countries. The World Health Organization (WHO), with 30 times the global incidence seen in the past 50 years, has estimated 50–100 million dengue infections annually. DENV actually poses a significant public health problem and about 40% of the world’s population is in danger of being infected with dengue (Guo et al., 2017). WHO reports that every year 2.5 billion people live in dengue-infested environments (Levoir and Goo, 2020). There is a wide range of illness that can be established, from subclinical to moderate serious type of dengue fever. A common side effect of serious dengue disease is relapse and there is a risk that it will lead to death if ignored.

© 2021 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The transmission of DENV is vulnerable to all biotic causes, including bacteria, vectors and infected humans. Weather is one of the abiotic factors that has a major effect on plant growth. The DENV are members of the family Flaviviridae and genus Flavivirus. These little viruses contain single-strand RNA as their own genome. A virion consists of a nucleocapsid which is enclosed in a phospholipid envelope. There are six structural protein genes and a lot of non-structural genes that make up dengue virus. Among non-structural proteins, envelope glycoprotein and NS1 plays a very important role which is a 45 kDa in size and has hemagglutination activity, and neutralization reactivity (Organization, 2011).

DENV is a single-strand virus with four serotypes: DENV 1–4. Individuals with DENV immunity are subject to only specific serotypes. Various serotypes cannot be defended against due to antigenic heterogeneity. As a result, DENV-2 infection was usually found in DENV-1 infections. Humans with dengue disease can range from mild fever to fatal bleeding (Ko et al., 2020). The number of Dengue cases is predicted to rise in the future as a result of factors such as climate change, globalization, viral development, insufficient economic and political assistance, and limited resources for effective control measures (Polwiang, 2020). Dengue expert consensus groups have declared that dengue is one entity that has a variety of signs and a variety of ailments. Dengue infection can cause an asymptomatic phase, in which patients have no symptoms, followed by the dengue fever (DF), Dengue hemorrhagic fever (DHF) and finally, dengue shock syndrome (DSS) (Hadinegoro, 2012). As shown by the expansion of DENV-1 into Hawaii in 2002, this virus was continued to spread. The latest outbreak of dengue fever in Hawaii is the first in the state since World War II ended. Over a duration of 1 year, 122 cases of serologically confirmed dengue fever were reported on the Hawaiian Islands of Maui, Oahu, and Kauai. And Culex virus is slow-moving unlike the fast-moving Aedes aegypti (Halstead, 2007). The total number of dengue infections is about ~13 billion (12825 128–13 130258) in Indian individuals aged 5–45 years. The lower figure of this study is a little less than the figure by Bhatt and colleagues (Bhatt et al., 2013), but being based on nationally representative data, this number is more accurate (Murhekar et al., 2019; Wilder-Smith and Rupali, 2019). Dengue infects about 1 billion people every year and causes 250,000 deaths. It is well known that early and specific diagnosis of DHF and DSS leads to lower mortality and morbidity (Peters, 1998).

Dengue fever in the western and southern regions of Saudi Arabia and is considered to be the key cause of seasonal fever in Jeddah and Makkah, two major infectious epidemics that occurred in 2011 and 2013. Dengue fever has also been documented in other regions of Saudi Arabia, such as Al-Madinah in 2009 and Aseer as well as Jizan in 2013 (Ajlan et al., 2019). Limited domestic studies have been performed in the Saudi Arabia but there is no study which was carried out for the past 9 years specifically in the Jazan region. So, the aim of this study was to estimate the epidemiological profile for the past decade and to detect the novelties in circulating virus serotypes in the Jazan region. Simultaneously, 100 diagnosed Dengue patients were screened with RT-PCR analysis.

2. Materials and methods

2.1. Study area

To investigate the retrospective cross-sectional study, Jazan (Gizan) region was opted from Saudi Arabia. Jazan is Southwest Saudi Arabia’s main Red Sea port. The region is located around 60 km north of the Yemen-Saudi Arabia border. Jazan is situated between the latitudes of 16°–12 and 18°–25 north. It is bordered in the south by the Arabic republic of Yemen, which has a total size of around 22,000 km² and a population of 1.5 million people. Around 30% of the population lives in six big towns, with the remaining spread throughout nearly 3500 villages. Jazan had a land area of 409 km² in 2018. The recovery of coastal lands and active sabkhas to the west of the old town was part of the coast’s urban growth. The monthly average temperatures range from 25.8 °C in January to 33.4 °C in July. The humidity levels fluctuate between 55% and 72.5% on average. From August to October, the rainy season begins with an average monthly rainfall of 77 mm and 56.7 mm. Jazan is one of the richest agricultural regions in the Kingdom, which is distinguished both by the quality and variety of its output. It is remarkable for its coffee bean, grain and fruit production such as apples, bananas, lemons, mangoes, oranges, papayas, and plums. The city of Jazan comprises 17 governors and 32 towns. Although these locations differ in heights and location, their demographic, agricultural, educational, cultural, housing, healthcare and environmental qualities are virtually same (Abo-Elimagd et al., 2018; ALSHEIKH et al., 2017; Pankratz et al., 2021).

2.2. Data collection

Dengue data was obtained in this study from 17 different hospitals including Al-Emis, Al-Hayat, Abu Arish, Al-Quwwat, Ahd Almursalat, Ateildabi Aleamm, Al-Mousam, Baysh, Bani Malik, Dharab, Dammad, Fida, Farasan, King Fahd Hospital, Mohammed Bin Naser, Sabia, Samta and Al-Tawal Hospitals and 19 different nationalities, including Saudi Arabia, such as Afghanistan, Bangladesh, China, Egypt, Eretia, Ethiopia, Filipino, India, Indonesia, Kenya, Palestine, Pakistan, Syria, Sri Lanka, Soman, Sudan, Turkey and Yemen between 2012 and 2020 in the Jazan city of the health affairs. General information such as age, gender, nationality, hospital name, and DENG test results were obtained. All the data was obtained from health affairs from Ministry of Health in the Jazan city.

2.3. Sample collection

In this study, 100 DEND positive samples were collected from 17 hospitals in Jazan. One mL of serum was collected from 4 mL of coagulant blood, and the separated serum was stored in the freezer for later examination.

2.4. RNA extraction

The genomic RNA was extracted from the stored serum sample using the EasyMag viral RNA purification kit. The magnetic beads technology allows for the purification of high-quality nucleic acids that are devoid of proteins, nucleases, and other contaminants. Extracted RNA was measured with NanoDrop with the spectrophotometer to measure both the quality and quantity of RNA in the extracted 100 serum samples. All the genomic RNA was finalized to 30 ng in each sample and used for the further RT-PCR analysis (Khan et al., 2015).

2.5. RT-PCR analysis

Early and specific identification of dengue virus genome in human serum samples is aided by real-time RT-PCR. One-step RT-PCR is a rapid, sensitive, and easy approach for diagnosing dengue serotypes. Using SYBR green, RT-PCR was carried out with 25ul of reaction using the fast-track diagnostics dengue differentiation kit which consists of 2ul of probes and 1ul of primers mix for DENV 1–4, 10ul of RT-PCR master mix including buffer(1ul) along with
1ul of each positive and negative controls. The complete reaction was carried out with adding 7ul of distilled water.

2.6. Statistical analysis

Clinical data was obtained as mean ± standard deviation between the collected samples (Khan et al., 2019). For the DENG samples, raw data will be either as positive or negative, there was one specimen which was found positive only after performing the RT-PCR.

3. Results

3.1. Sample collection analysis

In this cross-sectional study, we have enrolled 6637 subjects which were screened for DENG fever in the southwest region of Jazan in the Saudi Arabia. This study data was collected for 9 years i.e., 2012–2020. Fig. 1 explains the enrollment of the subjects between 2012 and 2020 in 17 different hospitals in the Jazan city. In 2012, 2.3% (n = 153) patients were diagnosed for DENG fever. In 2013 (3.1%, n = 206), 2014 (2.3%, n = 153), 2015 (7.1%, n = 469), 2016 (3.0%, n = 197), 2017 (8.5%, n = 567), 2018 (7.1%, n = 470), 2019 (42.7%, n = 2834) and 2020 (23.9%, n = 1588). The maximum number of enrolled cases were documented in 2019 (42.7%) and then 2020 (23.9%). Between 2012 and 2014, 2.3% were the minimum cases were enrolled.

3.2. Age and gender analysis

In this epidemiological study, the age range was starting from day 1 of birth to till 100 years of age. The mean age of 6637 subjects was 30.02 ± 18.01 of years. The age was categorized as shown in the Fig. 2. The number of subjects were in the age range between 0 and 10 years were 1040 subjects (15.7%), followed by 11–20 years (15.4%, n = 1020), 21–30 years (23.5%, n = 1561), 31–40 years (20.8%, n = 1378), 41–50 years (12%, n = 797), 51–60 (6.9%, n = 460), 61–70 (3.4%, n = 223), 71–80 (1.5%, n = 97), 81–90 (0.8%, n = 54) and 91–100 (0.1%, n = 7). In this study, we have recruited 62.8% (n = 4166) of males and 37.2% (n = 2471) of females (Fig. 3).

3.3. Nationality

In this study, 24 nationalities were included in the Saudi Arabia. The majority of three-fourth participants were Saudis (73.6%), followed by Yemen (13.2%), India (2.7%), Egypt (2.2%), Bangladesh, Pakistan and Sudan (1.9%). The other country subjects such as Ethiopia (0.98%), Nepal (0.51%), Filipino (0.29%), China (0.18%), Eritrea and Syria (0.14%), Palestine (0.08%), Indonesia (0.06%), Nigeria, Somalia, Sri Lanka and Turkey 0.05%, Kenya (0.03%), Afghanistan, Jordan, Morocco and Uganda (0.02%). All the details were shown in Fig. 4.

3.4. Positive and negative

Among the 6637 enrolled subjects, 56.6% (n = 3757) of them was found to be positive and remaining 43.4% (n = 2880) of the subjects were DENG negative. The details have been expressed in the Fig. 5.
3.5. Year-wise positive cases

The Fig. 6 describes the positive cases obtained in this study by the year-wise. In the year 2012, around 4.1% (n = 153) of DEND cases were registered, in 2013 (2.3%, n = 86), 2014 (2.2%, n = 83), 2015 (7.2%, n = 270), 2016 (3.8%, n = 142), 2017 (8.5%, n = 321), 2018 (4.6%, n = 173), 2019 (44%, n = 1654) and 2020 (23.3%, n = 875) were registered. The maximum cases were registered in the year 2019 by 44% and 2.2% were the minimum cases obtained in 2013.

3.6. Age and gender wise positive cases

Among 6637 enrolled cases, 3757 was found to be positive among the enrolled study. The number of subjects were in the age range between 0 and 10 years were 331 subjects (8.8%), followed by 11–20 years (16.6%, n = 623), 21–30 years (25.3%, n = 951), 31–40 years (23.6%, n = 885), 41–50 years (13.9%, n = 524), 51–60 (7.2%, n = 269), 61–70 (3.1%, n = 118), 71–80 (1.0%, n = 36), 81–90 (0.5%, n = 17) and 91–100 (0.1%, n = 3). All the details were shown in Fig. 7. Among the genders, males were found to be 61.8% (n = 2322) and 38.2% (n = 1435) was documented in the females as shown in the Fig. 8.

3.7. Nationality wise positive cases

The majority of three-fourth participants were Saudis (73.1%), followed by Yemen (13.2%), India (2.8%), Egypt (2.6%), Bangladesh (2.4%), Pakistan (2.2%) and Sudan (1.6%). The other country subjects such as Nepal (0.6%), Ethiopia (0.45%), China (0.2%), Filipino (0.2%), Eritrea (0.13%), Syria and Palestine (0.08%) Indonesia, Kenya, Srilanka and Turkey have 0.05% positive cases among the nationalities, Nigeria, Somalia, Afghanistan, Jordan, and Uganda were documented with 0.03%. However, there was no documented cases in the morocco nationality. All the details were shown in Fig. 9.

3.8. Quality control analysis

In this study, we randomly selected 100 samples from the Jazan region suspected to be DEND virus based on symptoms, and we confirmed that 93% of participants were positive and 7% of them turned to be negative as described in Fig. 10.
4. Discussion

Dengue fever is a mosquito-borne viral infection that has been identified in urban and predominantly semi-urban areas. Dengue virus/DENV is the name given to the virus that causes dengue. One of the symptoms of DENV infection is flu-like sickness. DENV is an RNA flavivirus with a positive strand of RNA. Infected Aedes mosquitoes, particularly Aedes aegypti, transmit DENV to humans. In the majority of cases, dengue infection after an incubation period of between 2 and 7 days may lead to a broad range of clinical symptoms, ranging from mild, flu-like, fever in combination with severe headache, retro orbital pain, myalgia and arthralgia (called dengue fever), to the most severe types of leucopenia, thrombocytopenic (dengue hemorrhagic fever). The latter can progress to hypovolemic shock (dengue shock syndrome). DENV infection is a leading cause of infection in tropical and subtropical climates, causing 50–100 million infections per year. DENV-1, DENV-2, DENV-3, and DENV-4 are four serotypes that are related yet distinct. Infection with one of the serotypes results in lifetime immunity to that serotype but not to another.

In this study, 42.7% of the maximum individuals were documented in 2019 prior to the COVID-19 pandemic. The affected participants ranged in age from 21 to 30 years old, with a prevalence rate of 23.5%. In our study, about two-thirds of the males were affected. In this study, more than 56% of the overall cases were positive, with 44% of the positive cases occurring in 2019. DENV cases were most common in people aged 21–30, accounting for 25.3% of all cases. The Saudi population had the highest incidence rate of 73.1% among the 19 ethnicities studied, and Uganda had the lowest prevalence rate of 0.03%. Previous studies in the kingdom from four different cities have indicated that males and youngsters are the major risk factors for DENV, and some global study results were inconclusive because females were regularly impacted in Australia, France, and Mexico (Alhaeli et al., 2016). Our findings were also consistent with the previous studies of Saudi population, since two-thirds of males are strongly affected in youngsters (21–30 years of age). A prior study from India found that 40% of the patients were under the age of five years (Mishra et al., 2015) and this study was corroborated by the Pandey et al study (Pandey et al., 2012), which had a 2:1 male to female ratio for probable DENV cases.

The relationship between DENV and Saudi Arabia dates back more than three decades; before, Saudi Arabia was considered to be a dengue-free country. The first dengue case (DENV-2) in the kingdom was registered in 1994 at Jeddah in 289 humans and subsequently several outbreaks have occurred across the Saudi Arabia (Fakeeh and Zaki, 2001). DENV-2 was identified first, followed by DENV-1 and DENV-3 in the kingdom. The DENV disease outbreak spread in Jeddah during the winter season of 2005–2006, and the
ministry of health (MOH) suppressed the disease with sophisticated measures. DENV was spread in Al-Madinah city in 2008 with DENV-1 and DENV-2 serotypes, and the MOH later confirmed 3350 DENV cases with a death rate of 4.6 (El-Badry et al., 2014). The prevalence of DENV was documented in different regions in the Kingdom ranging from 0.1 to 56.9% and 11.42% was in Sudan (Eldigaili et al., 2020). In our study, serotypes 2 and 3 were the predominant DENV serotypes between 2012 and 2020. Our study agreed with the Khan et al. studies performed in the Pakistani population (Khan et al., 2020).

Several real-time PCR-based approaches for DENV detection have been described in the last decade. These assays focused on the 3’UTR, NS5, core, and envelope gene sequences. Although these methods are useful for DENV serotyping, they may not be cost effective for routine diagnoses, as only a small proportion of samples are favorable in the DENV RNA during the non-endemic season and only about 50% of samples during active transmission can be positive in the DENV RNA season (Gurukumar et al., 2009). Identifying the molecular subtypes of these viruses, as well as any other novel genotypes, is contingent on determining their molecular makeup. RT-PCR has numerous advantages over traditional approaches for diagnosing acute dengue virus infection, such as virus isolation, anti-DENV IgM detection, and conventional RT-PCR (Dos Santos et al., 2008).

Previously, IgM and IgG antibodies were used to diagnose dengue infection, but NSI antigen has now been replaced for more accurate diagnosis. Couple of studies have been reported to be pleased with their findings (Carg et al., 2011; Nikam et al., 2015). A study was compared the NSI antigen and RT-PCR assays in DENV diagnosis, and the study results verified that both NSI antigen and RT-PCR assays can be adopted for rapid analysis (Ahmed and Broor, 2014). In our study, we have obtained the RT-PCR tests rather than the antibodies. Serum samples was commonly used between antibody and RT-PCR tests. Limited previous studies have used RT-PCR analyses to detect the Dengue fever/DENV virus (Barbham et al., 2006; Grobusch et al., 2006; Sasmono et al., 2014). There have been a few studies that have used saliva and urine samples to perform RT-PCR assays (Hirayama et al., 2012; Mizuno et al., 2007; Poloni et al., 2010; Van den Boschke et al., 2015).

The strength of this study was we have enrolled the epidemiological data obtained between 2012 and 2020 in the Jazan premises from the ministry of Health sector in Jazan city. Additionally, we have screened the 100 samples randomly selected for the diagnosis of DENV based on the symptoms and performed the RT-PCR assay rather than the antigen tests. One of the limitations of this study was we couldn’t perform the IgG or IgM or NSI antigen tests and opting 100 samples for screening of RT-PCR was another limitation of this study.

5. Conclusion

This epidemiological study has confirmed the prevalence of DENV was increased drastically since 2012 to 2020. High number of cases were registered prior to the Pandemic. The screening for DENV was performed with RT-PCR assay and NSI antigen should also be implemented to cross-check the results which was previously performed with RT-PCR analysis.

Potential impact of conflict on public health programs and new disease pattern can be observed. Specific response and efforts from health affairs should be taken into consideration to limit the influence of armed conflict consequences on health as crisis management policy. The great compatibility between clinical diagnosis and laboratory confirmed test showed the progress in the accumulative experience for the health care workers in the region and highlighted the need to adopt a new protocol to diagnose the missing and underreporting infection. Dengue poses a risk during rainy season when large populations have high degree of contact with the vehicle of the infection that play a role in distribution of the infection in certain months and places.

Until now there is now appearance of DENV-4 in Jazan region and DENV-2 is leaderboards of the scene followed by DENV-1 then DENV-3.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We are grateful towards the Deanship of Scientific Research of Jazan University, KSA for supporting and funding this research project No. (REC93/8-D397).

References

Abo-Elmagd, M., Saleh, A., Affif, G., 2018. Evaluation of radon related parameters in environmental samples from Jazan city, Saudi Arabia. J. Radiat. Res. Appl. Sci. 11 (1), 104–110.
Ahmed, N.H., Broor, S., 2014. Comparison of NS1 antigen detection ELISA, real time RT-PCR and virus isolation for rapid diagnosis of dengue infection in acute phase. J. Vector Borne Dis. 51, 194.
Ajar, B.A., Alafif, M.M., Alawi, M.M., Akbar, N.A., Aldigs, E.K., Madani, T.A., Marks, F., 2019. Assessment of the new World Health Organization’s dengue classification for predicting severity of illness and level of healthcare required. PLoS Negl. Trop. Dis. 13, e0007144.
Alhaeli, A., Bahkali, S., Ali, A., Househ, M.S., El-Metwally, A.A., 2016. The epidemiology of Dengue fever in Saudi Arabia: A systematic review. J. Infection Public Health 9 (2), 117–124.
Alshehri, A.A., Daffalla, O., Noureldin, E., Mohamed, M., Shwani, K., Hobani, Y., Akbar, A., Alshehri, F., Assisi, A., 2017. Serotypes of dengue viruses circulating in Jazan region, Saudi Arabia. J. Egypt. Soc. Parasitol. 47 (2), 235–246.
Anoopkumar, A., Aneesh, E.M., 2021. Environmental epidemiology and neurological manifestations of dengue serotypes with special inference on molecular trends, virus detection, and pathogenicity. Environ. Dev. Sustainability, 1–23.
Barbham, T.M., Chung, Y.K., Tang, K.F., Ooi, E.E., 2006. The performance of RT-PCR compared with a rapid serological assay for acute dengue fever in a diagnostic laboratory. Trans. R. Soc. Trop. Med. Hyg. 100 (2), 142–148.
Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J. M., Brownstein, J.S., Ho, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaensch, T., Wint, G.R.W., Simmons, C.P., Scott, T.W., Farrraj, J.J., Hay, S.L., 2013. The global distribution and burden of dengue. Nature 496 (7446), 504–507.
dos Santos, H.W.G., Poloni, T.R.R.S., Souza, K.P., Muller, V.D.M., Tresmeschin, F., Nali, L.C., Fantinatti, L.K., Amarilla, A.A., Castro, H.L.A., Nunes, M.R., Carne, B.M., Vazconcellos, P.F., Badra, S.J., Figueiredo, I.T.M., Aquino, V.H., 2008. A simple one-step real-time RT-PCR for diagnosis of dengue virus infection. J. Med. Virol. 80 (8), 1426–1433.
El-Badry, A.A., El-Beshbishi, H.A., Ali, A.K.H., Hejijn, A.M., El-Sayed, W.S.M., 2014. Molecular and seroprevalence of imported dengue virus infection in Al-Madinah, Saudi Arabia. Comp. Clin. Pathol. 23 (4), 861–868.
Eldigaili, M.H., Abubaker, H.A., Khalid, F.A., Abdallah, T.M., Adam, I.A., Adam, G.K., Babiker, R.A., Ahmed, M.E., Haroun, E.M., Aradaib, I.E., 2020. Recent transmission of dengue virus and associated risk Facors among residents of Kassala state, eastern Sudan. BMC Public Health 20, 1–9.
Fakher, M., Zaki, A., 2001. Virologic and serological surveillance for dengue fever in Jeddah, Saudi Arabia, 1994–1999. Am. J. Tropical Med. Hygiene 65, 764–767.
Garg, A., Garg, J., Rao, Y., Upadhyay, G., Sakhuja, S., 2011. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J. Infect. Dis. Immunity 3, 85–89.
Grobusch, M.P., Niedrig, M., Gobels, K., Klipstein-Grobusch, K., Teichmann, D., 2006. Evaluation of the use of RT-PCR for the early diagnosis of dengue fever. Clin. Microbiol. Infect. 12 (4), 395–397.
Guo, C., Zhou, Z., Wen, Z., Liu, Y., Zeng, C., Xiao, D., Ou, M., Han, Y., Huang, S., Liu, D., Ye, X., Zou, X., Wu, J., Wang, H., Zeng, E.Y., Jing, C., Yang, G., 2017. Global epidemiology of dengue outbreaks in 1990–2015: a systematic review and meta-analysis. Front. Cell. Infect. Microbiol. 7. https://doi.org/10.3389/fcimb.2017.00117.
Gurukumar, K.R., Priyadarshini, D., Patil, A.A., Bhagat, A., Singh, A., Shah, P.S., Cecilia, D., 2009. Development of real time PCR for detection and quantitation of dengue viruses. Virol. J. 6 (1), 10. https://doi.org/10.1186/1743-422X-6-10.
Hadinengoro, S.R.S., 2012. The revised WHO dengue case classification: does the system need to be modified? Paediatrics int. Child Health 32 (sup1), 33–38.

A. Alshahi, A. Marwan, N. Fatima et al. Saudi Journal of Biological Sciences 29 (2022) 204–210
