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ORIGINAL ARTICLE

Molecular and seroprevalence of imported dengue virus infection in Al-Madinah, Saudi Arabia

Ayman A. El-Badry · Hesham A. El-Beshbishy · Khalil H. Al-Ali · Ahmed M. Al-Hejin · Wael S. M. El-Sayed

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Abstract Recently, urban outbreaks of dengue virus (DENV) have occurred in western areas of Saudi Arabia; Jeddah and Makkah, neighbouring cities to Al-Madinah, where there is growing population traffic. DENV activity has not previously been reported in Al-Madinah. Molecular detection of DENV RNA using type-specific single-step real-time RT-PCR and seroprevalence of anti-DENV antibodies using ELISA was reported among Al-Madinah population for the first time through a cross-sectional study from May 2008 to July 2009. A total of 351 febrile, clinically suspected patients were identified, and acute dengue infection was identified in 71 of them during the first week of onset of fever; 5 (1.4 %) by real-time reverse transcription PCR alone, 45 (12.8 %) cases by IgM-ELISA alone and 13 (3.7 %) by both, while 8 (2.3 %) cases were identified during the second week of fever by the presence of IgM-ELISA only. Anti-DENV IgG antibodies were not detected in any of the tested samples. Of the 71 cases, 55 were resident in Al-Madinah (37 Saudi and 18 non-Saudi); however, all of them were imported cases. DENV-1 and DENV-2 were identified in 7 and 11 cases, respectively, while DENV-3 and DENV-4 were not detected in any cases. It was observed that the middle-aged group was the most infected group. DENV anti-DENV IgM antibodies showed a positive correlation of high significance with the number of days with fever. Nationality and gender were found to be significant independent predictors.

Keywords DENV RNA · Real-time RT-PCR · Anti-DENV antibodies · Al-Madinah

A. A. El-Badry (✉)
Department of Medical Parasitology, Kasr Al-Ainy School of Medicine, Cairo University, Cairo, Egypt
e-mail: aelbadry@kasralainy.edu.eg

K. H. Al-Ali
Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, Al-Munawwarah, P.O. 30001, Al-Madinah, Saudi Arabia

H. A. El-Beshbishy
Department of Medical Laboratories Technology, Faculty of Applied Medical Sciences, Taibah University, Al-Munawwarah, P.O. 30001, Al-Madinah, Saudi Arabia

W. S. M. El-Sayed
Department of Biology, Faculty of Medicine, Taibah University, Al-Munawwarah, P.O. 30001, Al-Madinah, Saudi Arabia

A. M. Al-Hejin
Department of Biological Sciences Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

H. A. El-Beshbishy
Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt

W. S. M. El-Sayed
Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt

Introduction

Diseases vectored by insects are responsible for an average of 50–100 million deaths annually and are, therefore, the most lethal diseases; dengue is one of the most important (Jacob 2001). Dengue fever (DF) is a major public health problem in tropical and subtropical regions (Harris et al. 2000), it is one of the most rapidly increasing mosquito-transmitted infections worldwide, and it causes more illness and death in humans than any other arbovirus (Lam 1993; Gubler 2002). An estimated 500,000 cases of dengue haemorrhagic fever require

hospitalization each year, of which, a very large proportion are children (DeRoeck et al. 2003). Although it is widely identified in Southeast Asia, the true incidence is not well documented (WHO 1999, 2000).

In 1994, dengue virus (DENV) was isolated in Jeddah, Saudi Arabia, for the first time, this was followed by an outbreak in 2006, and since then, reports of sporadic cases continue to appear in Jeddah and Makkah, western Saudi Arabia. *Aedes aegypti* was the indigenous mosquito responsible for establishment of dengue fever virus. Three dengue serotypes were detected: DENV-1, DENV-2 and DENV-3 (Ghaznawi et al. 1997; Fakeeh and Zaki 2001; Fakeeh and Zaki 2003; Ayyub et al. 2006; Zaki et al. 2008; Khan et al. 2008). All four DENV serotypes usually co-circulate with genotypic variation occurring among isolates within a serotype (Baeumner et al. 2002; De Paula et al. 2004; Ito et al. 2004; Putonti et al. 2006).

Laboratory diagnosis of DENV infection is based on detection of anti-DENV antibodies and/or virus antigen, virus isolation, or molecular detection of DENV RNA (Vorndam and Kuno 1997; WHO 1997; Gubler 1998; Guzman and Kouri 2004; Kao et al. 2005). The anti-DENV IgM and IgG antibodies using ELISAs are currently the most useful methods for serological diagnosis. In primary acute cases, IgM antibodies appear 3–5 days after the onset of the illness and can persist for 3–5 months. Anti-dengue IgG antibody levels are comparatively low and appear after approximately 14 days. In secondary infection, the IgG antibodies are detectable at a higher level, and the IgM antibodies may appear (Innis et al. 1989; Vaughn et al. 1997). Serological tests are easy to use and able to accommodate a great number of samples, properties which are necessary when confronting an epidemic, but they cannot easily distinguish dengue types at the serotype level (De Paula et al. 2004; Kao et al. 2005).Conventional methods of detection and serotyping of DENV in infected individuals are made by virus isolation through cell culture-based test with serological identification (Guzman and Kouri 2004). However, they are labour intensive (requiring about a week), have low sensitivity and require a research laboratory. They were replaced by molecular diagnosis based on reverse transcription (RT)-PCR. RT-PCR has provided one of the most important steps in the molecular diagnosis of DENV. Several laboratories have published various RT-PCR protocols for DENV identification using conventional PCR and real-time RT-PCR (Henchal et al. 1991; Lanciotti et al. 1992; Guzman and Kouri 1996; Harris et al. 1998; Kuno 1998).

There is no specific treatment or vaccine for DF (WHO 1999; Gubler 2002), so early detection of the viral infection is critical to avoid potential epidemic episodes. Recent urban DENV outbreaks occurred in western areas of Saudi Arabia; Jeddah and Makkah, neighbouring cities to Al-Madinah which have intensive population traffic between them. DENV activity has not previously been reported in Al-Madinah. A cross-sectional molecular and seroprevalence study was conducted to determine the extent to which the Al-Madinah population has been exposed to dengue infections.

Patients and methods

Study population and sample collection

Blood samples were collected randomly from subjects attending health centres in Al-Madinah Al-Munawwarah, western Saudi Arabia. Venous blood samples (5 mL) were drawn and allowed to clot at room temperature for 1 h. Serum was separated and transferred into small tubes placed in an ice bucket to be transported at 4 °C to the laboratory at Taibah University where they were kept at −70 °C till further analysis. Study subjects were classified into two groups:

Group I Febrile group (351 people): Blood samples were collected from febrile patients with dengue-like symptoms, and their clinical data recorded. Serum samples were tested for anti-DENV IgM and IgG antibodies using a commercial ELISA l kit and for DENV RNA using one-step real-time RT-PCR and used to identify acute cases infected with dengue and to classify cases as either primary or secondary.
Group II Afebrile group (1,227 people): Blood samples were collected randomly from asymptomatic individuals in Al-Madinah and tested using ELISA for anti-DENV IgG antibodies and to estimate the seroprevalence of dengue. They were also tested for anti-DENV IgM antibodies which acted as the control group.

Anti-DENV IgM and IgG antibody detection

Briefly, 100 μL of diluted serum (1:100) was added in each test well in triplicate. ELISA micro-plate was covered and incubated for 60 min at 37 °C, then washed thrice with 300 μL 5 % sodium hypochlorite solution, and the remaining liquid was removed. One hundred microlitres of conjugate was added to all wells except the blank, followed by incubation at 37 °C for 30 min. Then, 100 μl of substrate was added to all wells, incubated at 17–25 °C for 15 min. Finally, 100 μl of Stop Solution was added to all wells. The absorbance was measured immediately using spectrophotometer at 450 nm. The sample was considered to be negative if the concentration was ≤9 U/mL and positive if concentration was ≥11 U/mL.

Molecular detection of DENV

RNA extraction

DENV RNA was extracted from serum samples using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany; cat.

no. 5052904) according to the manufacturer's instructions. In brief, each serum sample was mixed with 200 μl of working solution and 50 μl of proteinase K and incubated for 10 min. One hundred microlitres of isopropanol was then added, and the entire mixture was transferred to the spin column and centrifuged at 10,000 rpm for 1 min. The bound RNA was washed twice with wash buffer. Lastly, the RNA eluted in a final volume of 60 μL of buffer AVE and stored at −70 °C till further use. RNA purity was detected by measuring OD260/OD280 spectrophotometrically, a value of 2 indicated that the RNA was pure enough to conduct the real-time RT-PCR.

Real-time RT-PCR assay

The assay was performed using the DENV type-specific real-time RT-PCR commercial kit (ZJ Bio-Tech, Shanghai, China; DENV-1 (cat. no. ER-0063-01), DENV-2 (cat. no.ER-0063-02), DENV-3 (cat. no.ER-0064-03) and DENV-4 (cat. no. ER-0064-04)) in single-step procedure according to the manufacturer's instruction on the StepOne™ Real-Time PCR System (Applied Biosystems, USA).

The reaction was performed as a one-step real-time RT-PCR. The first step was RT. Possible PCR inhibition was identified by measuring the VIC/JOE fluorescence of the internal control. An external positive control (standard 1×10^7 copies per millilitres) allowed the determination of the gene load. All data were investigated through the use of step one plus software. The real-time RT-PCR was performed using the following program: 45 °C for 10 min for 1 cycle, followed by 95 °C for 15 min for 1 cycle, then 95 °C for 15 s, 60 °C for 60 s for 40 cycles. The fluorescence was measured at 60 °C.

Statistical analysis

Statistical analysis was carried out on selected variables (age, sex, nationality, presence of fever for 2–7 days, severe headache and clinical data), and correlations were performed using Statistical Package for the Social Sciences (SPSS for Windows Version 13) and PrizmPad V.4.0 software. Correlation was considered highly significant with $P<0.0001$.

Results

This molecular and seroprevalence study was, to our knowledge, the first study conducted in the Al-Madinah area. A total of 1,578 serum samples were collected from febrile patients (351) and afebrile (1,227) subjects attending Al-Madinah Al-Munawwarah health centres.

ELISA detected serum anti-DENV IgM antibodies in 18.8 % (66/351) of febrile patients, 14.8 % (52/351) of them were resident; 10 % (35/351) Saudi's (19 males and 16 females) and 4.8 % non-Saudi's (ten males and three females) (Table 1). Nationality of non-Saudi residents in Al-Madinah positive for DENV RNA and/or anti-DENV IgM and IgG antibodies are presented in Fig. 1. Anti-DENV IgG antibodies were detected in 16.5 % (260/1,578) of all screened study samples, none were positive for anti-DENV IgM antibodies. All were residents, 79.2 % (206/260) Saudi's (144 males and 92 females) and 20.8 % (54/206) non-Saudi's (36 males and 18 females).

Using real-time RT-PCR, DENV RNA was detected in 18 (5.1 %) febrile samples, 5 by RT-PCR alone and 13 by both ELISA and RT-PCR. Of these, 13 were resident in Al-Madinah (seven males and six females) and 8 were Saudi's (Table 1). DENV-1 was detected in seven cases, three Saudi's and four non-Saudi, while DENV-2 was detected in 11 cases, seven Saudi's and four non-Saudi. DENV-3 and DENV-4 were not detected in any case (Table 1 and Fig. 2).

All age groups were represented in this study as the age ranged from 2 to 88 years (mean, 37.02); the middle age group was the most representative group with 54.9 % of the study population. This was also true for positive cases, the middle age group was the most infected age group, and anti-DENV IgM antibodies, anti-DENV IgG antibodies and DENV RNA were detected in 55 % (143/260), 64.9 % (48/74) and 72.2 % (13/18), respectively (Fig. 3).

Figure 4 shows the days of fever of acute cases (had anti-DENV IgM antibodies and/or DENV RNA); most cases were detected in samples at the end of the first week. Anti-DENV IgM antibodies and DENV RNA detection showed significant correlation with days of fever, $r^2=0.2053$, $p<0.0001$ using Pearson analysis, Figs. 4 and 5 (P value <0.05).

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 centre of the Islamic World. Al-Madinah Al-Munawwarah, Makkah and Jeddah are visited annually by nearly five million Muslims from all over the world to perform pilgrimage (Hajj) and little pilgrimage (Umarh) 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).

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

Table 1 DENV and anti-DENV IgM antibody prevalence detected in the study subjects

			Febrile			
			+ve by IgM-ELISA alone	+ve by PCR alone	+ve by both (ELISA and PCR)	Total
Resident	Saudi	♂	16	1	3	20
		♀	13	1	3	17
		Total	29	2	6	37
	Non-Saudi	♂	10	1	2	13
		♀	3	0	2	5
		Total	13	1	4	18
	Total	♂	26	2	5	33
		♀	16	1	5	22
		Total	42	3	10	55
Non-resident	Saudi	♂	6	1	1	8
		♀	1	0	0	1
		Total	7	1	1	9
	Non-Saudi	♂	3	1	1	5
		♀	1	0	1	2
		Total	4	1	2	7
	Total	♂	9	2	2	13
		♀	2	0	1	3
		Total	11	2	3	16
Total	53	5	13	71		

than 50 % of infected individuals, orviral symptoms typical DF to a severe and fatal dengue haemorrhagic fever/dengue shock syndrome (Innis 1995; Gubler 1998; Endy et al. 2002). 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.

All laboratory-confirmed cases in this study were self-limiting DF cases or immune cases. All acute cases were imported cases and had visited an endemic area 2 weeks prior to the onset of the illness. A considerable proportion of the adult population in Al-Madinah Al-Munawwarah have been exposed to dengue infection as reflected by the prevalence of anti-DENV IgG seropositivity (16.5 %). However, seroprevalence is much lower than that from other cities in endemic countries, where the prevalence is 69% (Salvador, Brazil), 78 % (Delhi, India) and 66 % (Iquitos, Peru) (Kurukumbi et al. 2001; Reiskind et al. 2001; Teixeira et al. 2002). Simultaneously, an entomological survey was carried out, where identification of *A. aegypti* was recorded for the first time, and the prevalence, seasonal variations and *Aedes* population diversity in Al-Madinah were documented (El-Badry and Al-Ali 2010). The relatively herd immunity (IgG) of the population in Al-Madinah may explain the absence of dengue infection outbreak or epidemics in Al-Madinah Al-Munawwarah in spite of the presence of the *Aedes* vector.

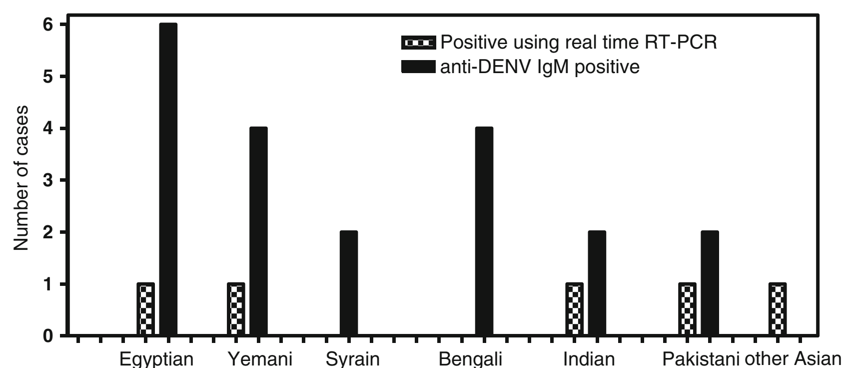

Fig. 1 Nationality of non-Saudi resident in Al-Madinah positive for serum DENV RNA using real-time RT-PCR and anti-DENV IgM antibodies using ELISA

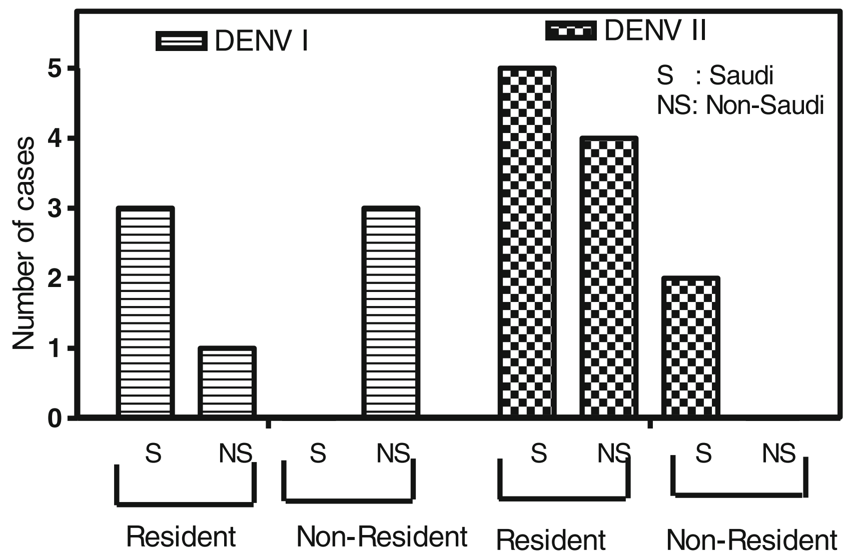

Fig. 2 DENV genotypes in study subjects in relation to their nationality and residency

However, the potential of outbreaks cannot be ruled out. The probability of dengue transmission will be low in an area regardless of the magnitude of measures of entomological risk if human herd immunity is high (Kuno 1998).

Diagnosis of DENV infection in this study and in clinics and laboratories worldwide is 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 (Schwartz et al. 2000), 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 (Chanama et al. 2004).

Cases of JE and YF do not exist in Al-Madinah Al-Munawwarah 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 Al-Madinah Al-Munawwarah large hospitals attract visitors and staff from different areas of Al-Madinah.

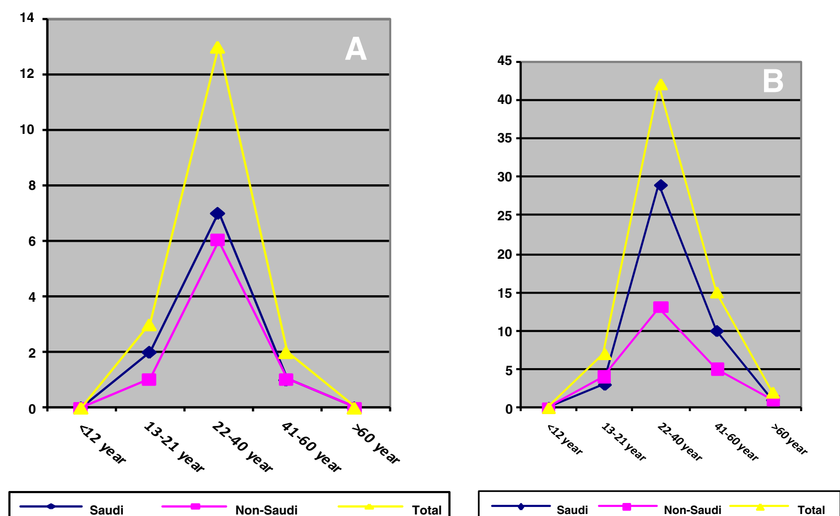

Fig. 3 Age groups of patients positive for **a** DENV RNA and **b** IgM serum anti-DENV antibodies

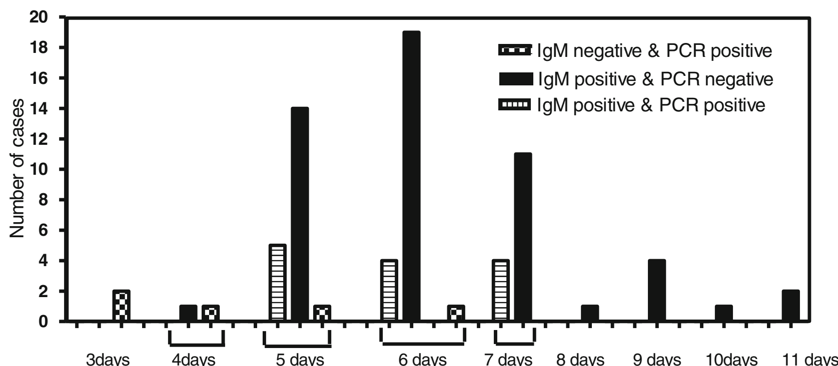

Fig. 4 Frequency of days of fever at time of testing in positive cases by ELISA for detection of anti-DENV IgM antibodies and/or RT-PCR

No previous data on the dengue prevalence situation in Al-Madinah are available, but a similar study conducted in Jeddah, Saudi Arabia reported anti-DENV IgG prevalence of 32 % (Fakeeh and Zaki 2001), Thailand showed 32 %, and Peru has an overall prevalence for anti-DENV IgG of 29.5 % (Reiskind et al. 2001).

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 (Groen et al. 2000).

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). RT-PCR-based diagnosis would be preferable for this purpose since it is positive early during the course of the disease and becomes negative toward the end of the febrile period when IgM becomes positive (Chan et al. 1998); thus, the two methods can be complementary. Several real-time PCR-based methods for the detection of DENV have been reported in the last decade. Serotype-specific real-time RT-PCR tests are able to detect and quantify DENV in the different kinds of samples (Poersch et al. 2005; Conceição et al. 2010).The quantitative aspect of real-time PCR is an evolutionary step in virology studies which allows disease severity to be related to viral load. Real-time RT-PCR has gradually replaced the virus isolation method as the new standard for the detection of DENV. It has many advantages over conventional PCR, including rapidity, ability to provide quantitative measurements, lower contamination rate, higher sensitivity, higher specificity and easy standardization (Kong et al. 2006).

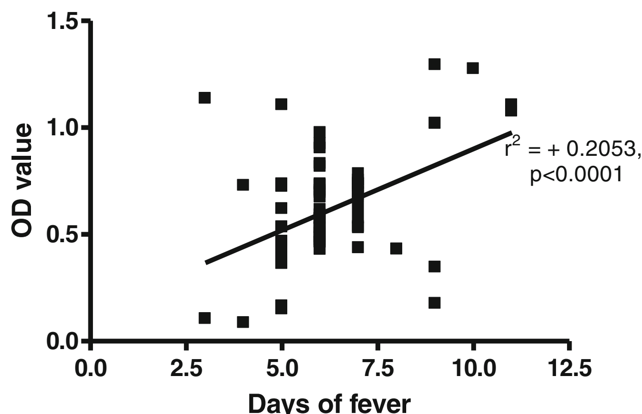

Fig. 5 The linear regression analysis of presence of fever (days) and the IgM OD absorbance value in patients suspected with DENV infection. The regression analysis was carried out using GraphPad Prism software V. 4.0 based on Pearson analysis showed significant positive r^2=+0.2053, P<0.0001

The pattern of distribution of DENV serotypes detected in this study population showed that DENV-2 was the most prevalent among Saudi patients (70 %) compared to 30 % for DENV-1, while none were DENV-3 or DENV-4 positive. A similar pattern was noticed in non-Saudi resident in Al-Madinah (Table 1 and Fig. 2).

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). 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).

All age groups were presented in this study; the middle age group is the most represented and the most DENV-infected age group. In the present study, a linear association of anti-DENV IgM antibodies prevalence with age was detected; this increased with age till middle age and then decreased (Fig. 3). This may result from a relatively stable transmission rate over the last decade. This result is in accordance with previous work that also showed an age-dependent increase of anti-dengue antibodies in exposed populations (Reiskind et al. 2001).If a serum sample is collected between days 1–3 of the disease in PD cases, both IgM and IgG antibody detection will give negative results. In these early days of a fever, a diagnosis will only be possible by RT-PCR or virus isolation. Samples collected between days4–7 are most frequently obtained for diagnostic purposes. In this interval, a combination of RT-PCR and IgM antibody assay may detect DF with a sensitivity

of 100 %. Obviously, the percentage increases with time, but a late diagnosis may be irrelevant for the clinician. A combination of IgM capture and indirect IgG ELISAs has been adopted by most laboratories for dengue serodiagnosis, and the IgM/IgG ratio can differentiate primary and secondary infections (Vaughn et al. 1997) even easier than haemagglutination inhibition test (WHO 1997).

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.

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References

Ayyub M, Khazindar AM, Lubbad EH, Barlas S, Alfi AY, Al-Ukayli S (2006) Characteristics of dengue fever in a large public hospital, Jeddah, Saudi Arabia. J Ayub Med Coll Abbottabad 18:9–13

Baeumner AJ, Schlesinger NA, Slutzki NS, Romano J, Lee EM, Montagna RA (2002) Biosensor for dengue virus detection: sensitive, rapid, and serotype specific. Anal Chem 74:1442–1448

Chan SY, Kautner IM, Lam SK (1998) The influence of antibody levels in dengue diagnosis by polymerase chain reaction. J Virol Methods 49:315–322

Chanama S, Anantapreecha S, A-nuegoonpipat A, Sa-gnasang A, Kurane I, Sawanpanyalert P (2004) Analysis of specific IgM responses in secondary dengue virus infections: levels and positive rates in comparison with primary infections. J ClinVirol 31:185–189

Conceição TM, Da Poian AT, Sorgine MHF (2010) A real-time PCR procedure for detection of dengue virus serotypes 1, 2, and 3, and their quantitation in clinical and laboratory samples. J Virol Methods 163(1):1–9

De Paula SOD, Lima CDM, Torres MP, Pereira MR, da Fonseca BAL (2004) One-step RT-PCR protocols improve the rate of dengue diagnosis compared to two-step RT-PCR approaches. J Clin Virol 30:297–301

DeRoeck D, Deen J, Clemens JD (2003) Policymakers' views on dengue fever/dengue haemorrhagic fever and the need for dengue vaccines in four southeast Asian countries. Vaccine 22:121–129

El-Badry AA, Al-Ali KH (2010) Prevalence and seasonal distribution of dengue mosquito, *Aedes aegypti* (Diptera: Culicidae) in Al-Madinah, Saudi Arabia. J Entomol 7(2):80–88

Endy TP, Chunsuttiwat S, Nisalak A, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA (2002) Epidemiology of in apparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. Am J Epidemiol 156:40–51

Fakeeh M, Zaki AM (2001) Virologic and serologic surveillance for dengue fever in Jeddah, Saudi Arabia, 1994–1999. AmJTrop Med Hyg 65(6):764–767

Fakeeh M, Zaki AM (2003) Dengue in Jeddah, Saudi Arabia, 1994–2002. Dengue Bull 27:13–18

Ghaznawi HI, Al-Khateeb TO, Akbar N, Afifi H, Nasser A (1997) Surveillance for dengue fever in Jeddah. East Mediterr Health J 3:567–570

Groen J, Koraka P, Velzing J, Copra C, Osterhaus ADME (2000) Evaluation of six immunoassays systems for the detection of dengue virus specific IgM and IgG antibodies. ClinDiagnImmun 6:1–5

Gubler DJ (1998) Dengue and dengue hemorrhagic fever. Clin Microbiol Rev 11(3):480–496

Gubler DJ (2002) The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res 33(4):330–342

Guzman MG, Kouri G (1996) Advances in dengue diagnosis. Clin Diagn Lab Immunol 3:621–627

Guzman MG, Kouri G (2004) Dengue diagnosis, advances and challenges. Int J Infect Dis 8(2):69–80

Harris E, Roberts TG, Smith L, Selle J, Kramer LD, Valle S, Sandoval E, Balmaseda A (1998) Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. J Clin Microbiol 36:2634–2639

Harris E, Videa E, Perez L (2000) Clinical epidemiologic and virologic features of dengue in the 1998 epidemic in Nicaragua. AmJTrop Med Hyg 63:5–11

Henchal EA, Polo SL, Vorndam V, Yaemsiri C, Innis BL, Hoke CH (1991) Sensitivity and specificity of a universal primer set for the rapid diagnosis of dengue virus infections by polymerase chain reaction and nucleic acid hybridization. AmJTrop Med Hyg 45:418–428

Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswadi V, Suntayakorn S, Puttisri P, Hoke CH (1989) An enzyme linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. AmJTrop Med Hyg 40:418–427

Innis BL (1995) Dengue and dengue hemorrhagic fever. In: Porterfield JS (ed) Exotic Viral Infections-1995. Chapman & Hall, London, United Kingdom, pp 103–146

Ito M, Takasaki T, Yamada K, Nerome R, Tajima S, Kurane I (2004) Development and evaluation of fluorogenic Taq Man reverse transcriptase PCR assays for detection of dengue virus types 1 to 4. J ClinMicrobiol 42(12):5935–5937

Jacob M (2001) Scientists target return of infectious diseases. Gen Eng News 21(10):11–80

Khan NA, Azhar EI, El-Fiky S, Madani HH, Abuljadial MA, Ashshi AM, Turkistani AM, Hamouh EA (2008) Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. Acta Trop 105(1):39–44. doi:10.1016/j.actatropica.2007.09.005

Kao CL, King CC, Chao DY, Wu HL, Chang GJ (2005) Laboratory diagnosis of dengue virus infection: current and future perspectives in clinical diagnosis and public health. J MicrobiolImmunol Infect 38:5–16

Kuno G (1998) Universal diagnostic RT-PCR protocol for arboviruses. J Virol Methods 72:27–41

Kong YY, Thay CH, Tin TC, Devi S (2006) Rapid detection, serotyping and quantitation of dengue viruses by Taq man real-time one-step RT-PCR. J Virol Methods 138:123–130

Kurukumbi M, Wali JP, Broor S (2001) Seroepidemiology and active surveillance of dengue fever/dengue haemorrhagic fever in Delhi. Indian J Med Sci 55:149–156

Lam SK (1993) Rapid dengue diagnosis and interpretation. Malays J Pathol 15:9–12

Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV (1992) Rapid detection and typing of dengue viruses from clinical

samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol 30:545–551

Poersch C, Pavoni D, Queiroz M, de Borba L, Goldenberg S, dos Santos C, Krieger M (2005) Dengue virus infections: comparison of methods for diagnosing the acute disease. J ClinVirol 32 (4):272–277

Putonti C, Chumakov S, Mitra R, Fox GE, Willson RC, Fofanov Y (2006) Human-blind probes and primers for dengue virus identification Exhaustive analysis of subsequences present in the human and 83 dengue genome sequences. FEBS J 273:398–408

Reiskind MH, Baisley KJ, Calampa C, Sharp TW, Watts DM, Wilson ML (2001) Epidemiological and ecological characteristics of past dengue virus infection in Santa Clara, Peru. Trop Med Int Health 6:212–218

Schwartz E, Mileguir F, Grossman Z, Mendelson E (2000) Evaluation of ELISA-based sero-diagnosis of dengue fever in travellers. J ClinVirol 19:169–173

Teixeira M, Barreto G, Costa ML (2002) Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. Trop Med Int Health 7:757–762

Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A (1997) Dengue in the early febrile phase: viremia and antibody responses. J Infect Dis 176:322–330

Vorndam V, Kuno G (1997) Laboratory diagnosis of dengue virus infections. In: Gubler DJ, Kuno G (eds) Dengue and dengue hemorrhagic fever. CAB International, New York, N.Y., pp 313–334

WHO (1997) Dengue haemorrhagic fever: Diagnosis, treatment, prevention and control, 2nd edn. WHO, Geneva, Switzerland

WHO (1999) Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Scientific working group on dengue. Meeting report, Geneva, Switzerland, 3–5 April 2000. Geneva: WHO, 2000

WHO (2000) Guidelines for Surveillance and Mosquito Control. Sec. ed. WHO Regional Office Edu. In Action Series 8–12

Wilder-Smith A, Schwartz E (2005) Dengue in travellers. NEJM 353 (9):924–932

Zaki A, Perera D, Jahan SS, Cardosa MJ (2008) Phylogeny of dengue viruses circulating in Jeddah, Saudi Arabia: 1994 to 2006. Trop Med Int Health 13(4):584–592