

Original Research Article

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Analysis of Dengue Fever among Patients Attending Dutse General Hospital in Jigawa State, Nigeria

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ABSTRACT

This study was conducted from February 2018 to July 2018 among patients attending Dutse General Hospital. The study was aimed at determining the analysis of DENV fever among patients and describes the month-wise trend of the disease. A total of 390 blood serum samples were collected and DENV specific IgM and flavivirus IgG antibodies were determined by in-house enzyme linked immunosorbent assay (ELISA). Out of 390 febrile cases, 54 (13.9%) were found to be positive for anti-DENV IgM. Among the 54 dengue positive cases, 37 (68.5 %) were primary DENV infection and 17 (31.5%) were secondary DENV infection. The most affected age group was 36-45 years (20.4%) and least affected group being 6-15years (8.3%). Prevalence in difference age groups was statistically significant ($p = 0.021$). Primary DENV fever was common among the age group between 36-45 years while secondary dengue affected mostly the age group 26-35 years. In terms of primary DENV infection against secondary DENV infection, it was observed that infants (<1 year) were the most affected but this was not statistically significant ($p = 0.057$). The relationship between gender and DENV infections was not statistically significant ($p = 0.936$). Although, females aged between 26-35 years ($p = 0.010$) and males aged above 46 years ($p = 0.012$) were the most affected with DENV infection. Month-wise distribution of DENV infection was observed in February (20.0%) with least occurrence in July (4.7%). The association between the month and occurrence of disease was not statistically significant ($p = 0.325$). The present study has reported 13.9% prevalence of Dengue virus infections as the cause of acute undifferentiated fever among febrile patients in Mombasa County. Thus, calls for government attention to develop resources at hospital laboratories for early dengue diagnosis and management of patients, coupled with general awareness among the public and constant vigilance by the health care officials could help in combating dengue.

Keywords

Dengue fever,
ELISA, DENV,
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Introduction

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serum samples were collected and DENV specific IgM and flavivirus IgG antibodies were determined by in-house enzyme linked immunosorbent assay (ELISA). Out of 390 febrile cases, 54 (13.9%) were found to be positive for anti-DENV IgM. Among the 54 dengue positive cases, 37 (68.5 %) were primary DENV infection and 17 (31.5%) were secondary DENV infection. The most affected age group was 36-45 years (20.4%) and least affected group being 6-15years (8.3%). Prevalence in difference age groups was statistically significant ($p = 0.021$). Primary DENV fever was common among the age group between 36-45 years while secondary dengue affected mostly the age group 26-35 years. In terms of primary DENV infection against secondary DENV infection, it was observed that infants (<1 year) were the most affected but this was not statistically significant ($p = 0.057$). The relationship between gender and DENV infections was not statistically significant ($p = 0.936$). Although, females aged between 26-35 years ($p = 0.010$) and males aged above 46 years ($p = 0.012$) were the most affected with DENV infection. Month-wise distribution of DENV infection was observed in February (20.0%) with least occurrence in July (4.7%). The association between the month and occurrence of disease was not statistically significant ($p = 0.325$). The present study has reported 13.9% prevalence of Dengue virus infections as the cause of acute undifferentiated fever among febrile patients in Mombasa County. Thus, calls for government attention to develop resources at hospital laboratories for early dengue diagnosis and management of patients, coupled with general awareness among the public and constant vigilance by the health care officials could help in combating dengue.

Dengue is the most rapidly spreading mosquito-borne viral disease with an estimated incidence of 390 million cases per years (Simmons *et al.*, 2012; Bhatt *et al.*, 2013). It is

regarded as the most important arboviral disease worldwide (Gubler, 2011a) and it is estimated that every year between 2.5-3.6 billion people in over 125 endemic countries are at risk including 120 million travelers to these regions (Gubler, 2002a; Guzman and Kouri, 2002). About 2 million cases evolve to dengue hemorrhagic fever and about 20,000 may culminate to death (Gubler, 2002a, Shepard *et al.*, 2011). The first isolated case of dengue in Nigeria was in the 1960s (Carey *et al.*, 1971, Amarasinghe *et al.*, 2011), but dengue is not a reportable disease in this country with most cases often undiagnosed, misdiagnosed as malaria or referred to as fever of unknown cause. Dengue IgM seroprevalence of 30.8% was reported in Nigeria among febrile children Faneye *et al.*, 2013), while another study in the north of the same country among healthy children revealed a seroprevalence of 17.2% (Oladipo *et al.*, 2014). The finding from the later study needs to be interpreted with caution as it's not clear from the study when samples were collected considering it is well established that dengue IgM antibody production may last for a couple of weeks after infection (Schwartz *et al.*, 2000). Our recent survey of dengue IgG antibodies in Ibadan, Nigeria showed a seroprevalence of 73% among febrile patients age 4 – 82 years. A further investigation of samples for active dengue infection by non-structural 1 (NS1) antigen analysis revealed an NS1 seroprevalence of 35% (Oyero and Ayukekbong, 2014). These data are consistent with the fact that dengue is an endemic and emerging cause of fever in Nigeria. However, the disease is neglected, under recognized and under reported in Nigeria due to lack of awareness by health care providers and lack of prioritization by the public health authorities.

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Dengue virus (DENV) infection is one of the mosquito-borne viral diseases with a major impact on public health, globally (Guzman *et al.*, 2010). World Health Organization (WHO) data suggest that at least 100 countries are endemic of Dengue virus transmission. About 3.5 billion people, 55% of the world's population living in tropical and subtropical regions are at risk, with about 50 million DENV infections occurring annually and approximately 500,000 requiring hospitalization annually (WHO, 2009). The average case fatality rate is around 5%, and mainly among children and young adults (Beatty *et al.*, 2007). Dengue virus is a positive-sense, single-stranded RNA enveloped virus that comprises of four serotypes (DENV 1, 2, 3 and 4) that belong to family Flaviviridae and genus Flavivirus (ICTVdB, 2006). All four serotypes of DENV are serologically related, but antigenically distinct (Zanotto *et al.*, 1996). They produce a spectrum of clinical illnesses ranging from a

classical dengue fever (DF) to severe and potentially fatal complications known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (WHO, 2009). Dengue fever is marked by a sudden onset of high fever, severe headache and retro-ocular pain and myalgia. The symptoms and signs may be very similar to other viral infections. The distinctive characteristics of DHF and DSS consist of hemorrhagic manifestations, plasma leakage, and profound shock. Antibody dependent enhancement (ADE) of viral replication is considered as a major reason for severity of DHF and DSS (Halstead, 2002). However, other factors also might be associated with DHF, such as DENV genotype polymorphisms in human leukocyte antigen (HLA) and other host genes (i.e. transporter associated with antigen processing (TAP) and human platelet antigen (HPA) (Vaughn *et al.*, 2000; Soundravally and Hoti, 2007; Stephens, 2010). Peak DENV infection occurs after period of increased rainfall due to increased multiplication of the mosquito vector, *Aedes aegypti* (*Ae. aegypti*) (El-Badry and Al-Ali, 2010). *Aedes* mosquitoes shelter indoors and bite during the daytime. They are adapted to breed around human dwellings, in water containers, vases, cans, tires, and other discarded objects (El-Badry and Al-Ali, 2010). *Ae. albopictus* is also the vector for DENV which contributes significantly to transmission in Asia and whose presence is spreading in Latin American countries (Roiz *et al.*, 2008). Dengue outbreaks have also been attributed to *Ae. polynesiensis* and *Ae. scutellaris*, but to a lesser extent (Rodhain and Rosen, 1997). Early diagnosis of DENV infection is important for proper treatment of DHF and DSS to avoid fatal outcome. Currently, several dengue vaccine candidates are in an advanced stage of development (Morrison *et al.*, 2010). For example, Sanofi Pasteur's ChimeriVax-DENV vaccine has recently entered phase 3 clinical testing (Guy *et al.*, 2010; Collier and Clements, 2011).

Statement of the problems

Dengue virus infection is a complex disease with symptoms being difficult to distinguish from other common febrile illnesses during acute phase and can progress from a mild, non-specific viral disease to severe cases characterized by thrombocytopenia, hemorrhage manifestations and hemo-concentration due to plasma leakages. Majority of febrile illnesses in Mombasa County are treated as presumptive malaria, often without proper medical examination and a laboratory diagnosis. Therefore, many patients with fever are designated as having fever of unknown origin or malaria and remain without a laboratory diagnosis even if they fail to respond to antimalarial drugs. This situation is generally due to lack of affordable diagnostic reagents. The scenario indicates that many cases of DENV infections are undiagnosed or even misdiagnosed. Additionally, presence of dengue vector *Aedes aegypti* in the coastal region of Kenya as reported by Mwangangi *et al.*, (2012). Individual exposure differences to dengue infective bites may be related to prevalence with specific demographic factors such as age and gender that have not been reported among febrile patients in the County of Mombasa.

Justification of the Study

Dengue is the most rapidly spreading mosquito-borne viral disease with an estimated incidence of 390 million cases per years (Simmons *et al.*, 2012; Bhatt *et al.*, 2013). It is regarded as the most important arboviral disease worldwide (Gubler, 2011a) and it is estimated that every year between 2.5-3.6 billion people in over 125 endemic countries are at risk including 120 million travelers to these regions (Gubler, 2002a, Guzman and Kouri, 2002). About 2 million cases evolve to dengue hemorrhagic fever and

about 20,000 may culminate to death (Gubler, 2002a; Shepard *et al.*, 2011).

Arboviruses are widespread in Nigeria considering that the mosquito vectors responsible for the transmission of dengue, yellow fever, chikungunya (*Aedes* spp) and those responsible for malaria (*Plasmodium* spp) are well established in this country. Dengue co-infection with other arbovirus infections is therefore not uncommon and has been described in Nigeria (Baba *et al.*, 2012). These co-infections might provide an opportunity for exchange of genetic materials and mutations resulting in the emergence of strains with fitness and enhanced disease severity. Antibody cross reactivity by viruses of the *flaviviridae* family may also affects accurate serological diagnosis. Early signs and symptoms of dengue are indistinguishable from those of other tropical disease fever like malaria and typhoid. In Nigeria where malaria is highly endemic; most cases of febrile illnesses are likely to be treated as presumptive malaria (Amexo *et al.*, 2004). We recently reported that 10% of malaria patients in Ibadan, Nigeria had active dengue infection. Further evaluation of dengue IgG seroprevalence among malaria patients revealed that all the malaria patients in the study were positive for dengue IgG antibodies suggestive of a past dengue infection and consistent with the endemicity of dengue virus in the region (Oyero and Ayukekbong, 2014).

The number of reported dengue cases has increased since the 1980s due to factors such as unplanned urbanization, lack of surveillance and vector control, poor public health, international travel and virus and vector evolution (Guzman and Kouri, 2002, Gubler, 2011b). Understanding risk factors to infection is important for public health control programs. The evaluation of male-female difference in infection rates for instance has been difficult to discern. Three independent

studies from dengue epidemics in Singapore and India found that the risk of infection in males was two times higher in females (Goh *et al.*, 1987; Agarwal *et al.*, 1999; Wali *et al.*, 1999). A few studies in South America including out recent study in Nigeria reveal that both sexes are equally affected (Vasconcelos *et al.*, 1993; Rigau-Perez *et al.*, 2001; Oyero and Ayukekbong, 2014). Taken together, a comprehensive evaluation of sex difference in infection rate requires well-designed studies that would take into consideration both biological and social factors that drive dengue transmission in the population.

The contribution of climate change to DENV transmission has been investigated previously and the incidence and, in particular epidemics of dengue has been common during the rainy season (Hales *et al.*, 1996; Keating, 2001). The availability of favorable breeding grounds for the mosquito vector enhances the spread of DENVs. Due to water requirements for breeding, mosquito densities speak during the wet season, resulting in an increase in the number of dengue cases during this period (Hales *et al.*, 2002).

The poor drainage system and inadequate waste disposal in most Nigeria cities results in the presence of stagnant water bodies and water collected in waste metal containers and vehicle tires. These media serve as breeding sites for the mosquito vectors which are the agents of DENV transmission (Baba and Talle, 2011). The increase in the number of susceptible individuals in these areas also enhances the risk of human to mosquito transmission and vice versa. Therefore, due to the nature of the route of infection, those at greatest risk of infection are those in regular exposure to the mosquito vector. A high IgG seroprevalence has be reported among adults >40 years of age compared to those younger than 40 years of age which is consistent with

increased in vector exposure with age (Oyero and Ayukekbong, 2014).

Significance of study

Exposure to the dengue virus generally occurs in the infantile to juvenile period among residents in dengue endemic areas, and the prevalence of DENV infection increases with age and reaches its peak before adolescence. Collecting information on the prevalence among persons with febrile illness would be an initial step in determining the extent of dengue infections.

This will help the physicians to consider possibility of dengue cases when handling febrile patients, thereby proper management of the dengue patient to avoid fatal complications. Dengue prevalence is usually attributed to gender related differences in exposures, as gender roles and exposures change over the human lifespan. Examining both age and gender will provide prevalence of dengue stratified data that will help on targeting specific preventive measures.

Additionally, the study findings will deliver effective communication and coordination to the government and non-governmental partners, and the community to implement policy on adequate infection prevention practices and improve vector control programmes to reduce the dengue burden in the County.

The main objectives of this study to determine the prevalence of DENV infection by age and gender of among febrile patients in Jigawa State. And also to determine the proportion of primary and secondary DENV infection among febrile patients in Jigawa State.

Dengue viral infection

Dengue virus (DENV) infection is an acute

febrile illness, which occurs after an incubation of 4-10 days. Infection parity is known to be a critical factor of disease severity. Primary DENV infection with any of the four DENV serotypes is believed to elicit lifelong immunity against that serotype, but confers partial or transient immunity against other serotypes. Cross-reactive, but sub-neutralizing DENV-reactive IgG acquired by a previous heterotypic serotype infection may enhance DENV infectivity which may result in higher viral burden and contribute to induced disease severity. Heterologous secondary DENV infections have been associated with large, clinical outbreaks of Dengue hemorrhagic fever or Dengue shock syndrome (DHF/DSS), where severe dengue occurs most frequently in children (WHO, 1997).

Clinical manifestations

Most DENV infections are asymptomatic, but may result in a wide spectrum of disease that differs in severity from mild undifferentiated fever, the classical DF (Guha-Sapir and Schimmer, 2005), to the potentially fatal complications known as DHF and DSS (Figure 1). Clinical presentation in both children and adults may vary in severity depending on the immune status, age and the genetic background of the patient (WHO, 2009).

Dengue Fever

Most patients display mild fever or remain asymptomatic. However, symptomatic infection presents as classic dengue fever (DF) with an incubation period of 4 to 10 days. The clinical features of DF frequently depend on the age of the patient (Hammond *et al.*, 2005). Children are often asymptotically infected with DENV but may demonstrate several clinical syndromes. Infants and young children most often present with an undifferentiated

febrile illness accompanied by a maculopapular rash seen on the trunk and inside of the arms (George and Lum, 1997). Older children and adults typically present with classic DF characterized by an acute sudden onset saddleback fever, severe headache, nausea and vomiting, myalgia, retro-orbital pain, an early maculopapular rash, low grade thrombocytopenia and hepatomegaly (Henchal and Putnak, 1990). Patients with DF recover in two to seven days and suffer no short- or long-term sequelae of illness. The virus disappears from bloodstream at approximately the same time that the fever dissipates (Rothman, 1999).

Dengue Hemorrhagic Fever

Dengue Hemorrhagic Fever (DHF) usually follows a secondary dengue infection. In infants, it may follow a primary infection due to maternally acquired dengue antibodies (Halstead *et al.*, 2002). The clinical course of DHF is divided into three phases, namely, febrile, critical, and convalescent phases (Figure 4). The febrile phase begins with sudden onset of fever accompanied by generalized constitutional symptoms and facial flush. The fever is high grade (usually $>38.5^{\circ}\text{C}$), intermittent, and associated with rigors. The fever lasts for 2-7 days and then falls to normal when the patient either recovers or progresses to the plasma leakage phase (CDC, 2012a; Srikiatkachorn *et al.*, 2007). Some patients remain ill despite normalization of temperature and therefore progress to DHF. Onset of plasma leakage is characterized by tachycardia and hypotension. The patient sweats, becomes restless, and has cold extremities. In less severe cases, the changes are minimal and transient, reflecting a mild degree of plasma leakage. Most patients recover from this stage spontaneously or after a short period of fluid and electrolyte replacement. In severe cases with high plasma leakage, patients may develop full-blown

circulatory shock characterized by prolonged capillary refill time and narrow pulse pressures (WHO, 2009). During the phase of plasma leakage, pleural effusions and ascites are common. Pericardial effusions may also be seen. Myocarditis is associated with increased morbidity and mortality. Fever and hemo-concentration due to plasma leakage is most commonly observed before the subsidence of fever and the onset of shock (Kalayanarooj *et al.*, 2002).

Dengue Shock Syndrome

Dengue shock syndrome (DSS) is associated with almost 50% mortality. After a certain level of plasma leakage, the compensatory mechanisms become insufficient and blood pressure drops rapidly. Pulse pressure drops below 20 mmHg and symptoms of hypovolemic shock develop; sudden collapse, cool clammy skin, rapid weak pulse, circumoral, easy bruising and bleeding (hematemesis, melena, epistaxis), and myocarditis. Warning signs include severe abdominal pain, vomiting, irritability and somnolence, fall in body temperature and severe thrombocytopenia (Gibbons and Vaughn, 2002). Patients die from multi-organ failure and disseminated intravascular coagulation. Most patients remain fully conscious to the terminal stage. The duration of shock is short and the patient rapidly recovers with appropriate supportive therapy. DSS may be accompanied by encephalopathy caused by metabolic and electrolyte disturbances (Gurugama *et al.*, 2010).

Mosquito vectors

All the known vectors of DENV are mosquitoes belonging to genus *Aedes* (Ae.), subgenus *Stegomyia* (Figure 2). The species involved in transmission include *Ae. aegypti* usually in an urban environment and globally exists in tropical area. However, *Ae. albopictus* is present in Asia and the Pacific.

Ae. polynesiensis only exists in the Pacific (Rodhain and Rosen, 1997). The life cycle of a mosquito consists of four separate stages: egg, larva, pupa and adult (Figure 3), the first three stages requiring an aqueous environment. The duration of the developmental stages depend on the environment's temperature, water and availability of food at the larval stage. For *Ae. aegypti*, it takes 8-10 days at room temperature (Gubler, 1997). Adult male mosquito feed on flower nectar and juices of fruits for flight energy. The female requires a blood meal for egg development. Human blood is preferred and the ankle area is a favoured feeding site (Monath, 1994). *Aedes aegypti* female mosquito is highly anthropophilic (Huber *et al.*, 2008) and prefers to feed during the day - two hours after sunrise and few hours before sunset is the most appropriate time, although they feed all day indoors and on overcast days. Female *Ae. aegypti* mosquito shows a preference for laying their eggs in domestic containers, but may also use rainwater-accumulating containers present in peridomestic environments (Wongkoon *et al.*, 2007; El-Badry and Al-Ali, 2010). Its adaptation to human habitats and its desiccation-resistant eggs have allowed it to flourish in urban centers. They have a life span of 8 to 15 days and flight range for females is about 30 to 50 meters per day. These mosquitoes are unique in that they feed on more than one person per gonadotropic cycle and will resume feeding on a second individual if interrupted (El-Badry and Al-Ali, 2010).

Dengue Virus Transmission Cycles

Two transmission cycles are known for DENV, one of them involving non-human primates (monkeys) and jungle mosquitoes, referred to as the sylvatic cycle, and the second being the urban cycle that involves *Ae. aegypti* - human - *Ae. aegypti* which is most important transmission cycle that causes huge outbreaks in the tropics (Gubler and Meltzer,

1999) (Figure 4). The life cycle of DENV involves a replication step in both mosquito and human hosts. Infected humans are the main carriers and multipliers of the virus, serving as a source of the virus for uninfected mosquitoes (Monath, 1994). The virus circulates in the blood of infected humans for two to seven days and at approximately the same time patient develops fever. Uninfected *Aedes* mosquitoes acquire the virus when they feed on an individual during this period (Monath, 1994).

Once a mosquito has fed on a viremic human, the virus replicates in the arthropod mid-gut and disseminates to the salivary glands within 8-12 days. Following dissemination to the salivary glands, female *Aedes* mosquitoes are able to transmit DENV to new hosts. However, for the virus infection to be sustained in the vector mosquito, virus titer in the human host should exceed $10^5 - 10^7$ virus particles per ml (Monath, 1994). The vector itself is thought to function as an important biological filter for maintaining the virus titers at high level (Monath, 1994). In periods of low virus transmission, the DENV may survive through transovarial transmission from parent to progeny and possibly also between mosquitoes sexually (Khin and Khan, 1983). Direct person-to-person transmission has not been documented. Although, a few case reports have been published on transmission of DENV through exposure to DENV-infected blood, organs, or other tissues from blood transfusions, solid organ or bone marrow transplants, percutaneous and mucous membrane contact with dengue-infected blood (De Wazieres *et al.*, 1998; Chen and Wilson, 2004; Tan *et al.*, 2005; Wilder-Smith *et al.*, 2009).

Materials and Methods

Study site

This study was conducted at the Dutse

General Hospital (DGH) that provides the health care services to the local people and serves as a referral center to the entire County. The facility provides a variety of health care services through inpatient and outpatient departments under the units of medicine, surgery, gynecology, and other medical sub-specialties (e.g. pediatrics, obstetrics, and microbiology). DGH facility is located in the County of Nigeria.

Study design

This was a hospital-based prospective study conducted for a period of 6 months (February to July 2018).

Variables

The variable in the present study included age, gender, and month as independent variables, while dengue patient as dependent variable in this study.

Study Population

This study was performed among febrile patients seeking medical care at both the inpatient and outpatient departments.

Sample size

The sample size was 390 blood samples used for the study

Study procedure

Recruitment of patients

A trained study clinical officer recruited eligible patients and collected data at pediatric, outpatient and inpatient departments of CPGH. The study clinical officer introduced himself and explained to the parents and guardians the purpose of the study. Informed verbal and written consent was obtained from parents and guardians who

allowed their children to take part in the study (Appendix A).

The patients with the guardian were assured of confidentiality of the information. Participation in the study was on a voluntary basis.

Clinical and demographic data collection

A structured assessment form was used to obtain the clinical history regarding febrile illness including clinical symptoms and signs (Appendix B).

Blood sample collection procedure

The study clinical officer collected venous blood samples aseptically from the study participants as follows: The veins in the antecubital fossa or dorsum of the hand were identified and a tourniquet applied to make the veins visible. The area was then cleansed with an alcohol swab and allowed to air dry, 3-5ml of blood was drawn from each febrile patient using a sterile needle and syringe or vacutainer needle and serum separating tube (SST) (Becton Dickinson, SA).

Sample handling, transport and storage

The blood samples were centrifuged at 1,300 x g for 10 minutes at 4°C. A sterile, graduated, disposable transfer pipette was used to transfer serum into two sterile screw-capped cryotubes (1.5 ml per tube, Greiner Bio-One, Germany) and stored at -80°C until testing. The serum samples were collected and delivered to the Kenya Medical Research Institute, Production Department (KEMRI-PD) laboratories, Nairobi.

Laboratory procedures

Cell lines and virus strains

Aedes albopictus mosquito derived C6/36 cells and African green monkey kidney

derived Vero cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% v/v heat inactivated fetal bovine serum (FBS Sigma, USA) and 100units/ml penicillin, 100µg/ml streptomycin and 292 µg/ml L-glutamine (GIBCO), 0.1% non-essential amino acids (Gibco/Invitrogen, UK) and 2-3% Sodium bi-carbonate. C6/36 and Vero cells were cultured in 25 cm², 75 cm² tissue culture flasks (Nunc, Denmark) at 28°C and 37°C, respectively. The cell lines were passaged every 5-7 days. The cell monolayer was washed with 0.1% trypsin in 0.02% EDTA solution was added for 3 minutes at 28°C and 37°C, respectively. After addition of trypsin-EDTA solution, the flask was tapped to detach and disperse cells. Equal volume of culture medium was added to stop the enzyme activity and cell suspension centrifuged at 1,400 rpm for 4 minutes. The cell precipitate was re-suspended with growth medium and transferred into flasks. The DENV strains used in this study were: DENV-1 (Hawaii), DENV-2 (00St-22A), DENV-3 (SLMC-50), and DENV-4 (SLMC-318). All the strains were grown in the C6/36 cells at 28°C for 7-10 days and stored in aliquots at -80°C as seed virus stock until use.

Antigen production

Propagation and harvesting of the virus

Aedes albopictus clone C6/36 cell line was grown at 28°C in MEM with 10% FBS in Roux bottles. At 80% confluence, growth medium was removed and 1 ml of seed virus inoculated in each bottle, followed by 2 hours virus adsorption at 28°C. The inoculum was spread over the cell sheet every 20 minutes. Thereafter, maintenance medium was added to cell sheet and incubated at 28°C. After 14 days for DENV-1, 9 days for DENV-2, 12 days for DENV-3, and 10 days for DENV-4, the infected culture fluids (ICF) were collected in centrifuge bottles (Beckman Instruments, USA) and spun at 5000 rpm for 10 minutes at

4°C in a JLA-10.500 rotor (Beckman Instruments, USA) in Avanti J-26 XP centrifuge to remove cell debris. 3.7.2.2 Virus Concentration Using Jumbosep™ Centrifugal Devices a) Principle of the procedure Centrifugation up to 3,000 x g provides the driving force for filtration, moving sample toward the highly selective, low protein-binding Omega™ membrane. Molecules larger than the membrane's nominal molecular weight cutoff of 30K (MWCO-30K) are retained in the sample reservoir. Solutes and molecules smaller than the MWCO-30K of the membrane pass through the membrane surface into the membrane insert and through the filtrate port into the filtrate receiver (Pall Life Sciences, 2007).

Procedure

The procedure was performed by following manufacturer's instruction. The filtrate receiver was separate from the sample reservoir and membrane insert with the filtrate port facing down dropped into the sample reservoir (Figure 5). The sample reservoir was placed on a hard surface and membrane insert pressed down firmly to rest on the knobs at the bottom of the sample reservoir. Empty filtrate receiver was attached to the bottom of the sample reservoir, 60 ml of ICF was added to the sample reservoir and capped to prevent evaporation during centrifugation. The Jumbosep devices were placed in a swinging-bucket rotor (B438-29) that accepted standard 250 ml bottles and spun at 4,200rpm for 60 minutes at 4 °C in Tomy AX-311 versatile refrigerated centrifuge (Tomy, Japan). Jumbosep devices were removed at the end of spun time and sample reservoir separated from the filtrate receiver. Retentate was recovered by pouring off the retentate into pre-labeled 15 ml centrifuge tubes, a pipette tip sledged under the dislodged membrane insert and remaining retentate removed. The retentate fluid was then stored at -80 °C.

Sandwich ELISA to assay dengue antigen titer

The principle of Voller *et al.*, (1976) was used with some modifications (Bundo and Igarashi, 1985) A 96-well ELISA flat bottom plate was coated with anti-flavivirus IgG (20µg/ml) in coating buffer (0.05 M carbonate–bicarbonate buffer, pH 9.6, containing 0.02% sodium azide) at 4 °C overnight. The plate wells were blocked with Blockace (Yukijirushi, Japan) at room temperature (r.t). After washing with PBS-Tween 3 times, test samples, standard antigen, and negative control (MEM) were distributed in duplicate.

The plate was incubated at 37 °C and washed as above, and horseradish peroxide (HRPO)-conjugated anti-flavivirus IgG original (1:500 dilution in PBS-Tween) was distributed into all wells except blanks. Unbound conjugate was washed off as above, and the plate was incubated with substrate solution containing o-phenylenediamine dihydrochloride (OPD) and 0.05% hydrogen peroxide for 30 minutes at room temperature in the dark. The reaction was stopped by adding 1N sulfuric acid and optical density (OD) read at 492nm using Multiskan EX ELISA Reader (Thermo Scientific, China).

Preparation of dengue tetraivalent antigen

The DENV tetraivalent antigen for IgM capture ELISA was prepared by mixing equal titer of DENV 1, 2, 3 and 4 ICF to make 100 ELISA units. The mixture was aliquated in 10ml and stored at -80°C.

Dengue IgM-capture ELISA

An in-house DENV IgM-capture ELISA (in-house IgM ELISA) was carried out following the protocol described by Bundo and Igarashi, (1985). The 96-well flat-bottomed microplate (Maxisorp Nunc, Denmark) was coated with

anti-human IgM (μ -chain specific) 5.5 μ L/100 μ L/well (Cappel, Germany) and diluted with ELISA coating buffer in all wells except blanks. The plate was incubated at 37 °C for 1 h or at 4 °C overnight. All wells except the blank were blocked with 100 μ L of the original concentration of Blockace, and were incubated at room temperature (r.t) for 1 h. The reagents were removed from all of the wells by washing three times with phosphate buffered saline containing 0.05% Tween 20 (PBS-T). The test serum samples as well as positive and negative control sera at 1:100 dilutions in PBS-Tween were distributed in duplicate wells and incubated at 37°C for 60 minutes. After the reaction and washing, the DEN tetravalent antigen was distributed into the wells. The plate was incubated at 37 °C for 1h and washed as above. HRPO-conjugated anti-flavivirus IgG monoclonal antibody 12D11/7E8 (1:500 dilution in PBS-T and 10% Blockace) was added into all wells except blanks. After the incubation at 37 °C for 1h, the unbound conjugate was washed off and substrate solution containing OPD and 0.03% hydrogen peroxide was added to all wells to proceed in the dark at r.t. The reaction was stopped by adding 1N sulfuric acid and OD read at 492nm by ELISA plate reader. The ratio of the absorbance of the positive serum and negative serum (P/N) was calculated by dividing OD of serum sample by the OD of the negative control serum. The P/N ratio above or equal to 2.0 was considered positive.

Flavivirus indirect IgG ELISA

An in-house flavivirus IgG indirect ELISA modified by Inoue *et al.*, (2010) was used in detecting flavi IgG to determine primary and secondary dengue virus infections. In this modified procedure, purified Japanese encephalitis virus (JEV) antigen (strain: ML-17) was applied as an assay antigen (Bundo *et al.*, 1986). A 96-well microplate (Nunc International) was coated with 250ng/100 μ L

per well of virus antigen at 4 °C overnight. The wells were blocked with 100 μ L/well of Blockace at r.t for 1h, washed three times with PBS-T for 3 min each. Test sera were diluted at 1:1000 and standard serum was diluted by two serial from 1:100 upto 212 with PBS-T with 10% Blockace were each placed in duplicate wells and incubated at 37°C for 1h.

The plate wells were washed as above, and then reacted with 100 μ L/well of 1: 2000 diluted HRPO-conjugated anti-human IgG goat serum (American Qualex, CA) in PBS-T with 10% Blockace. After 1h incubation at 37 °C, the plates were washed as above and 100 μ L/well of substrate solution was added in each well. The substrate solution used was described in section 3.7.5. After 30 minutes incubation at r.t in the dark, the reaction was terminated by adding 100 μ L/well of 1 N sulphuric acid to each well. The OD was read at 492nm by ELISA plate Reader. The IgG titers of patient sera were determined from a positive standard curve. A sample titer \geq 1:52,000 was considered to be a DENV secondary infection, whereas a sample titer $<$ 1:52,000 was considered to be a DENV primary infection (Inoue *et al.*, 2010).

Serological definitions of DENV infection

- a) Laboratory-positive DENV infection case: a single positive anti-dengue IgM with P/N ratio equal to or greater than 2.0 according to the WHO case definition (Bundo and Igarashi, 1985; WHO, 2009).
- b) Primary DENV infection case: A laboratory-positive case in which the IgG-ELISA titer was $<$ 1:52,000 (Inoue *et al.*, 2010).
- c) Secondary DENV infection case: A laboratory-positive case in which the IgG-ELISA titer was \geq 1:52,000 (Inoue *et al.*, 2010).

Data storage and analysis

The data collected and generated in the laboratory was entered in excel spreadsheets in a password protected computer. The data was then converted to Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, USA) for analysis. The data for the IgG titers from the in-house IgG ELISA were expressed as the geometric mean. An analysis of variance (ANOVA) was used to compare geometric mean of the DENV cases across the age groups and months. A p-value less or equal to 0.05 ($p \leq 0.05$) was considered as statistically significant. Microsoft Excel was used to generate all graphs and table 1-4. The relationship of less than or equal to 5% between gender and dengue cases was analyzed using of Fishers exact tests between two categorical variables.

Antigen production

Propagation and harvesting of the virus

Aedes albopictus clone C6/36 cell line was grown at 28°C in MEM with 10% FBS in Roux bottles. At 80% confluence, growth medium was removed and 1 ml of seed virus inoculated in each bottle, followed by 2 hours virus adsorption at 28°C. The inoculum was spread over the cell sheet every 20 minutes. Thereafter, maintenance medium was added to cell sheet and incubated at 28°C. After 14 days for DENV-1, 9 days for DENV-2, 12 days for DENV-3, and 10 days for DENV-4, the infected culture fluids (ICF) were collected in centrifuge bottles (Beckman Instruments, USA) and spun at 5000 rpm for 10 minutes at 4°C in a JLA-10.500 rotor (Beckman Instruments, USA) in Avanti J-26 XP centrifuge to remove cell debris. 3.7.2.2 Virus Concentration Using Jumbosep™ Centrifugal Devices a) Principle of the procedure Centrifugation up to 3,000 x g provides the driving force for filtration, moving sample toward the highly selective, low protein-

binding Omega™ membrane. Molecules larger than the membrane's nominal molecular weight cutoff of 30K (MWCO-30K) are retained in the sample reservoir. Solutes and molecules smaller than the MWCO-30K of the membrane pass through the membrane surface into the membrane insert and through the filtrate port into the filtrate receiver (Pall Life Sciences, 2007).

Procedure

The procedure was performed by following manufacturer's instruction. The filtrate receiver was separate from the sample reservoir and membrane insert with the filtrate port facing down dropped into the sample reservoir (Figure 5). The sample reservoir was placed on a hard surface and membrane insert pressed down firmly to rest on the knobs at the bottom of the sample reservoir. Empty filtrate receiver was attached to the bottom of the sample reservoir, 60 ml of ICF was added to the sample reservoir and capped to prevent evaporation during centrifugation. The Jumbosep devices were placed in a swinging-bucket rotor (B438-29) that accepted standard 250 ml bottles and spun at 4,200rpm for 60 minutes at 4 °C in Tomy AX-311 versatile refrigerated centrifuge (Tomy, Japan). Jumbosep devices were removed at the end of spun time and sample reservoir separated from the filtrate receiver. Retentate was recovered by pouring off the retentate into pre-labeled 15 ml centrifuge tubes, a pipette tip sledged under the dislodged membrane insert and remaining retentate removed. The retentate fluid was then stored at -80 °C.

Sandwich ELISA to assay dengue antigen titer

The principle of Voller *et al.*, (1976) was used with some modifications (Bundo and Igarashi, 1985) A 96-well ELISA flat bottom plate was coated with anti-flavivirus IgG (20µg/ml) in coating buffer (0.05 M carbonate-bicarbonate

buffer, pH 9.6, containing 0.02% sodium azide) at 4 °C overnight. The plate wells were blocked with Blockace (Yukijirushi, Japan) at room temperature (r.t). After washing with PBS-Tween 3 times, test samples, standard antigen, and negative control (MEM) were distributed in duplicate. The plate was incubated at 37 °C and washed as above, and horseradish peroxidase (HRPO)-conjugated anti-flavivirus IgG original (1:500 dilution in PBS-Tween) was distributed into all wells except blanks. Unbound conjugate was washed off as above, and the plate was incubated with substrate solution containing o-phenylenediamine dihydrochloride (OPD) and 0.05% hydrogen peroxide for 30 minutes at room temperature in the dark. The reaction was stopped by adding 1N sulfuric acid and optical density (OD) read at 492nm using Multiskan EX ELISA Reader (Thermo Scientific, China).

Preparation of Dengue Tetraivalent Antigen

The DENV tetraivalent antigen for IgM capture ELISA was prepared by mixing equal titer of DENV 1, 2, 3 and 4 ICF to make 100 ELISA units. The mixture was aliquated in 10ml and stored at -80°C.

Dengue IgM-capture ELISA

An in-house DENV IgM-capture ELISA (in-house IgM ELISA) was carried out following the protocol described by Bundo and Igarashi, (1985). The 96-well flat-bottomed microplate (Maxisorp Nunc, Denmark) was coated with anti-human IgM (μ -chain specific) 5.5 μ L/100 μ L/well (Cappel, Germany) and diluted with ELISA coating buffer in all wells except blanks. The plate was incubated at 37 °C for 1 h or at 4 °C overnight. All wells except the blank were blocked with 100 μ l of the original concentration of Blockace, and were incubated at room temperature (r.t) for 1 h. The reagents were removed from all of the wells by

washing three times with phosphate buffered saline containing 0.05% Tween 20 (PBS-T). The test serum samples as well as positive and negative control sera at 1:100 dilutions in PBS-Tween were distributed in duplicate wells and incubated at 37°C for 60 minutes. After the reaction and washing, the DEN tetraivalent antigen was distributed into the wells. The plate was incubated at 37 °C for 1h and washed as above. HRPO-conjugated anti-flavivirus IgG monoclonal antibody 12D11/7E8 (1:500 dilution in PBS-T and 10% Blockace) was added into all wells except blanks. After the incubation at 37 °C for 1h, the unbound conjugate was washed off and substrate solution containing OPD and 0.03% hydrogen peroxide was added to all wells to proceed in the dark at r.t. The reaction was stopped by adding 1N sulfuric acid and OD read at 492nm by ELISA plate reader. The ratio of the absorbance of the positive serum and negative serum (P/N) was calculated by dividing OD of serum sample by the OD of the negative control serum. The P/N ratio above or equal to 2.0 was considered positive.

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then reacted with 100µl/well of 1: 2000 diluted HRPO-conjugated anti-human IgG goat serum (American Qualex, CA) in PBS-T with 10% Blockace. After 1h incubation at 37 °C, the plates were washed as above and 100µl/well of substrate solution was added in each well. The substrate solution used was described in section 3.7.5. After 30 minutes incubation at r.t in the dark, the reaction was terminated by adding 100µl/well of 1 N sulphuric acid to each well. The OD was read at 492nm by ELISA plate Reader. The IgG titers of patient sera were determined from a positive standard curve. A sample titer \geq 1:52,000 was considered to be a DENV secondary infection, whereas a sample titer $<$ 1:52,000 was considered to be a DENV primary infection (Inoue *et al.*, 2010).

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a) Laboratory-positive DENV infection case:

A single positive anti-dengue IgM with P/N ratio equal to or greater than 2.0 according to the WHO case definition (Bundo and Igarashi, 1985; WHO, 2009).

b) Primary DENV infection case:

A laboratory-positive case in which the IgG-ELISA titer was $<$ 1:52,000 (Inoue *et al.*, 2010).

c) Secondary DENV infection case:

A laboratory-positive case in which the IgG-ELISA titer was \geq 1:52,000 (Inoue *et al.*, 2010).

Data storage and analysis

The data collected and generated in the laboratory was entered in excel spreadsheets in a password protected computer. The data was then converted to Statistical Package for Social Science (SPSS) version 16.0 (SPSS Inc., Chicago, USA) for analysis. The data for

the IgG titers from the in-house IgG ELISA were expressed as the geometric mean. An analysis of variance (ANOVA) was used to compare geometric mean of the DENV cases across the age groups and months. A p-value less or equal to 0.05 ($p \leq 0.05$) was considered as statistically significant. Microsoft Excel was used to generate all graphs and tables. The relationship of less than or equal to 5% between gender and dengue cases was analyzed using of Fishers exact tests between two categorical variables.

Results and Discussion

Prevalence of dengue infection cases among febrile patients

During the study period, a total of 390 serum samples from febrile patients were tested for dengue antibodies using an in-house IgM-capture ELISA and indirect IgG ELISA. The patients were diagnosed for primary DENV infection, secondary DENV infection and non-dengue infection depending on antibody titer against DENV. Fifty four (13.9%) cases were confirmed as dengue infection while 336 (86.1%) cases were found to be non-dengue (Table 1).

Distribution of dengue positive cases by age

The age of all patients ranged from 2 month to 82 years. The mean age was 24.9 years, with median age of 25 years and standard deviation of 17.2 years. The age was grouped to capture the most vulnerable age group, as it is known that undifferentiated febrile illnesses is more often common among the pre-school children (1-5 years) and infants ($<$ 1 year), therefore may experience more severe clinical outcome after primary dengue infection (Guzman *et al.*, 2002; Hammond *et al.*, 2005). The highest affected group in the present study were patients aged between 36 - 45 years with 11 (20.4%) and least being children aged 6 - 15 year with 6 (8.3%). There was a significant

difference in occurrence of DENV infection by age groups ($p = 0.021$) as shown in Table 1.

Primary DENV infection was mainly observed among patients aged between 36-45 years with 8 (14.8%) and least in patients aged between 1-5 years with 3 (6.1%) (Table 1). The difference between primary DENV infection by age groups was statistically significant ($p = 0.049$). The highest secondary DENV infection was observed among patients aged between 26-35 years with 7 (7.8%) and infants (< 1 year) were the least affected 0 (0.0%) (Table 1). There was significance difference between secondary DENV infection by age groups ($p = 0.027$)

Proportion of primary verses secondary dengue cases

The highest primary DENV infection was observed among patients aged less than 1 year (100.0%) and the lowest among age group 1-5 years (50.0%) (Figure 6). Secondary DENV infection was highest in 1-5 years age group (50.0%), followed by 26-35 years age group (43.8%). There was no significant correlation between primary and secondary DENV infection ($p = 0.057$).

Distribution of dengue positive cases by gender

The distribution of 54 dengue positive cases between male and female were 28 (51.9%) and 26 (48.1%), respectively (Table 2). The male: female ratio was found to be 1:0.93. The relationship between gender and DENV infection was not statistically significant ($p = 0.936$). However, significant gender differences were observed in the age group 26-35 ($p = 0.010$) and ≥ 46 years ($p = 0.012$), respectively (Figure 7).

Out of 37 patients suffering from primary DENV infections, 51.4% were males and

48.6% were females (Table 3). The most affected groups were females aged between 26- 35 years with 44.4% ($p = 0.005$) and least cases of DENV infection noted in those above 46 years. Majority of males affected with primary DENV were above 36 years ($p = 0.019$), with the least prevalence observed in those less than 1 year. Gender differences in primary DENV infection was not statistically significant ($p = 0.911$).

Out of 17 patients that suffered from secondary DENV infection, 52.9% were males and 47.1% were females (Table 4). Males of age group 26-35 years were most affected at 33.3% and least affected group was aged less than 1 year at 0.0%. However, majority of females affected were aged between 26-35 years (50.0%) with least secondary dengue cases in age group < 1 year and ≥ 46 years (0.0%). Gender differences in secondary infection was not significant by age groups ($p = 0.737$).

Prevalence of dengue viral infection

The present study found a prevalence of dengue viral infections to be 13.9 % with 9.5 % as primary dengue cases and 4.4% as secondary dengue cases. The present study findings appeared to be higher as compared to study findings from the neighboring country (Cameroon) that reported 4.5% and 9.5% of dengue cases among the febrile patients (Vairo *et al.*, 2012; Hertz *et al.*, 2012). The present findings may be as due to the spatial diffusion of the virus and vector proliferation within the region. Since recent studies have reported dengue outbreaks. In 2010, Comoros, Mayotte and Tanzania reported outbreak of dengue fever caused by DENV-3 (Issack *et al.*, 2010; Sante-plus.org, 2010; Klaassen, 2010; Sissoko *et al.*, 2010). DENV infection has also been reported in Mogadishu, Somalia (WHO, 2011). Additionally, the heavy sea bound commercial traffic between western Africa and Indian sub-continent where all four

serotypes exist, and increased number of tourists and migrants from other endemic areas exposed the coastal region to vulnerability of imported dengue resulting to domestic spread of the disease (Matlani and Chakravarti, 2011).

During the present study, comparison between the different age groups revealed that adults were infected disproportionately to children. The most susceptible age group for DENV infection was 36-45 years and followed by 26-35 years suggesting that the individuals in these age groups were actively involved in outdoor activities that increased their chances of exposure to the infective DENV vector bite. Similar observations have been reported from South East Asia regions where adults were more affected than children (Tank and Jain, 2012).

Regarding children, a lower DENV infection was observed in age group < 1 year (9.1%) in respect to 1-5 years (12.2%). Since the vector *Ae.aegypti*, is a predominantly day biting outdoor vector, Children < 1 year were at a lower risk of dengue infection as they spend most of their time indoors, completely covered or sleep under bed nets unlike the children aged 1-5 year who were able to play and spend more time outdoors within and around the residential areas. A higher DENV infection was observed among children aged 1-5 years with DENV infection cases reaching a low point in the age group 6-15 years before rising again. Similar findings were observed from southeast India and Caribbean (Akram, 1998; Kumar *et al.*, 2013). The present findings may be explained by the fact that children aged 1-5 years spent most of their time either at home or at a nursery or kindergarten of which operates within residential areas or shop-houses. However, formal half-day schooling starts at the age of 6

years, often with afterschool extracurricular activities which lead to reduced exposure to mosquito bites among children aged 6-15 years. Although, secondary infection was highest in children aged 1-5 years, younger children aged < 1 year were at higher risk of severe dengue infection than children age 1-5 years. This was because of maternal antibody enhancement of disease, as maternal antibodies wanes from protective to enhancing levels (Halstead *et al.*, 2002; Hammond *et al.*, 2005).

Summary, conclusion and recommendation are as follows:

Dengue is an important emerging disease of the tropical and sub-tropical regions today. It is a complex disease whose symptoms are difficult to distinguish from other common febrile illnesses and can progress from a mild, non-specific viral disease to irreversible shock and death within a few hours. This makes the differential diagnosis problematic especially in the coastal region, where there is a high incidence of febrile illnesses such as typhoid fever and malaria. The study aimed at determining the prevalence of DENV infection. A total of 390 serum samples from febrile patients in a period of 6 months (February - July 2018). Dengue antibodies were tested using an in-house IgM-capture ELISA and indirect IgG ELISA. Fifty-four (13.9%) were found to be dengue cases with 37 (9.5%) as primary dengue and 17 (4.4%) as secondary dengue. Majority dengue infections were observed among 36-45 years. Both genders were equally susceptible to the DENV infection. Predominance among female aged 26 -35 years. Lastly, DENV infection occurred throughout the study period with peak dengue infection cases in February.

Table.1

Table 4.1 Prevalence of Dengue Virus Infection among Febrile Patients

Parameter	Febrile cases n (%)	Dengue cases (IgM +ve)		Total dengue cases n (%)	Non-dengue patients (IgM -ve) n (%)
		Primary infection IgG titer (<1:52,000) n (%)	Secondary infection IgG titer (≥1:52,000) n (%)		
Male	204	19 (9.3)	9 (4.4)	28 (13.7)	176 (86.3)
Female	186	18 (9.7)	8 (4.3)	26 (14.0)	160 (86.0)
Total	390 (100)	37 (9.5)	17 (4.4)	54 (13.9)	336 (86.1)
Age (years)					
<1	11	1 (9.1)	0 (0.0)	1 (9.1)	10 (90.9)
1 – 5	49	3 (6.1)	3 (6.1)	6 (12.2)	43 (87.8)
6 – 15	72	5 (6.9)	1 (1.4)	6 (8.3)	66 (91.7)
16 - 25	66	6 (9.1)	2 (3.0)	8 (12.1)	58 (87.9)
26 - 35	90	9 (10.0)	7 (7.8)	16 (17.8)	74 (82.2)
36 - 45	54	8 (14.8)	3 (5.5)	11 (20.4)	43 (79.6)
≥ 46	48	5 (10.4)	1 (2.1)	6 (12.5)	42 (87.5)
Total	390(100)	37 (9.5)	17 (4.4)	54 (13.9)	336 (86.1)

Table.2

Table 4.3 Distribution of primary dengue cases by age group and gender

Age group (years)	Gender		Total cases	p-value
	Male n (%)	Female n (%)		
< 1	1 (5.3)	0 (0.0)	1 (2.7)	
1 – 5	2 (10.5)	1 (5.6)	3 (8.1)	
6 – 15	3 (15.8)	2 (11.1)	5 (13.5)	
16 – 25	2 (10.5)	4 (22.2)	6 (16.2)	
26 – 35	1 (5.3)	8 (44.4)	9 (24.6)	0.005
36 – 45	5 (26.3)	3 (16.7)	8 (21.6)	
≥ 46	5 (26.3)	0 (0.0)	5 (13.5)	0.019
Total	19 (100)	18 (100)	37 (100)	0.911

Table.3

Table 4.3 Distribution of primary dengue cases by age group and gender

Age group (years)	Gender		Total cases	p-value
	Male n (%)	Female n (%)		
< 1	1 (5.3)	0 (0.0)	1 (2.7)	
1 – 5	2 (10.5)	1 (5.6)	3 (8.1)	
6 – 15	3 (15.8)	2 (11.1)	5 (13.5)	
16 – 25	2 (10.5)	4 (22.2)	6 (16.2)	
26 – 35	1 (5.3)	8 (44.4)	9 (24.6)	0.005
36 – 45	5 (26.3)	3 (16.7)	8 (21.6)	
≥ 46	5 (26.3)	0 (0.0)	5 (13.5)	0.019
Total	19 (100)	18 (100)	37 (100)	0.911

Table.4

Table 4.4 Distribution of secondary dengue cases by age and gender

Age group (years)	Gender		Total cases n (%)
	Male n (%)	Female n (%)	
< 1	0 (0.0)	0 (0.0)	0 (0.0)
1 – 5	2 (22.2)	1 (12.5)	3 (17.6)
6 – 15	1 (11.1)	0 (0.0)	1 (5.9)
16 – 25	1 (11.1)	1 (12.5)	2 (11.8)
26 – 35	3 (33.3)	4 (50.0)	7 (41.2)
36 – 45	1 (11.1)	2 (25.0)	3 (17.6)
≥ 46	1 (11.1)	0 (0.0)	1 (5.9)
Total cases	9 (100)	8 (100)	17(100)

Fig.1 Clinical Manifestations of DENV infection (WHO, 2009)

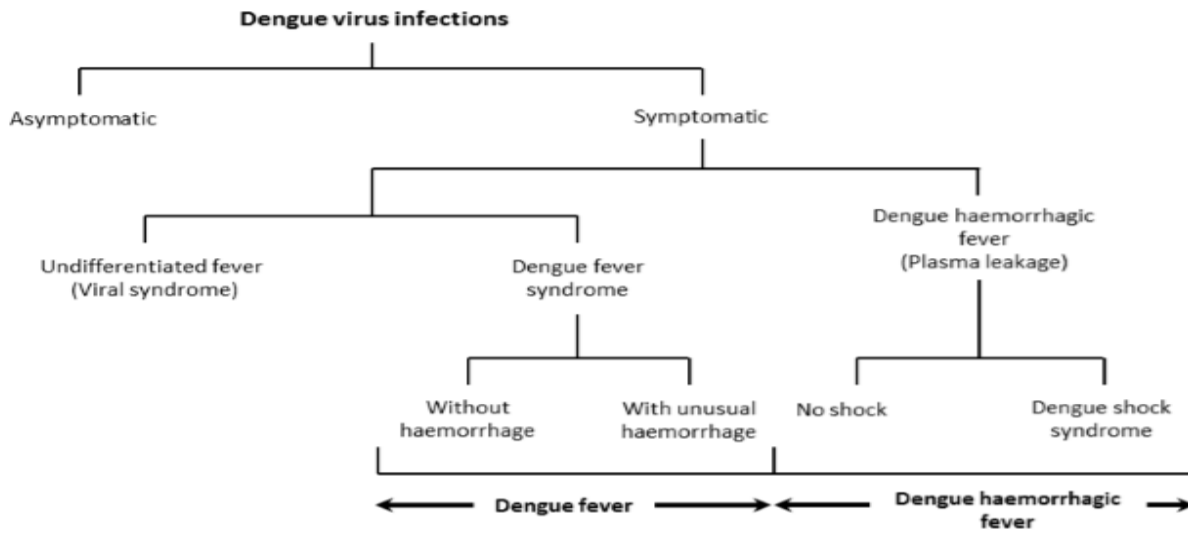


Fig.2 Mosquito vectors for DENV transmission (Rodhain and Rosen, 1997)



Fig.3 Life cycle of Aedes mosquito (Wongkoon et al., 2007)

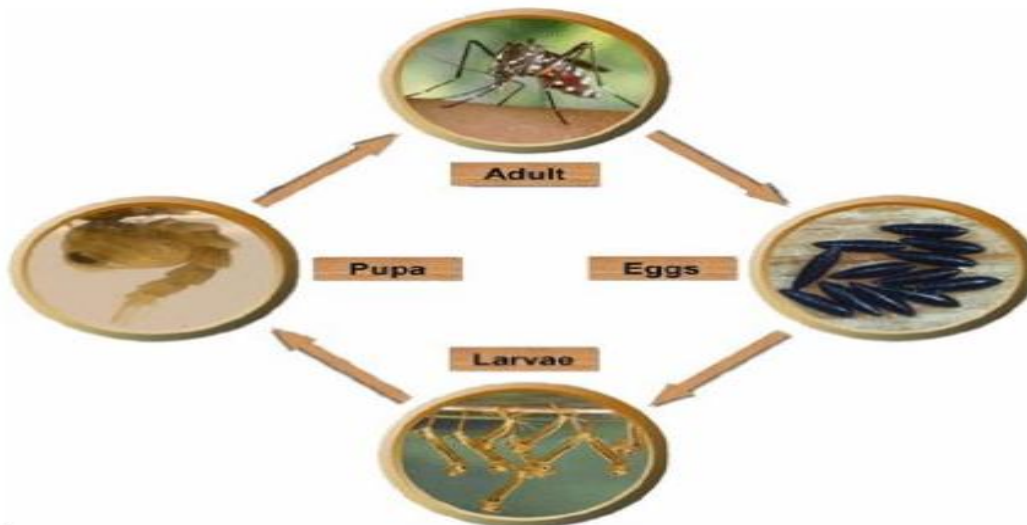


Fig.4 Transmission cycle of DENV (Whitehead et al., 2007)

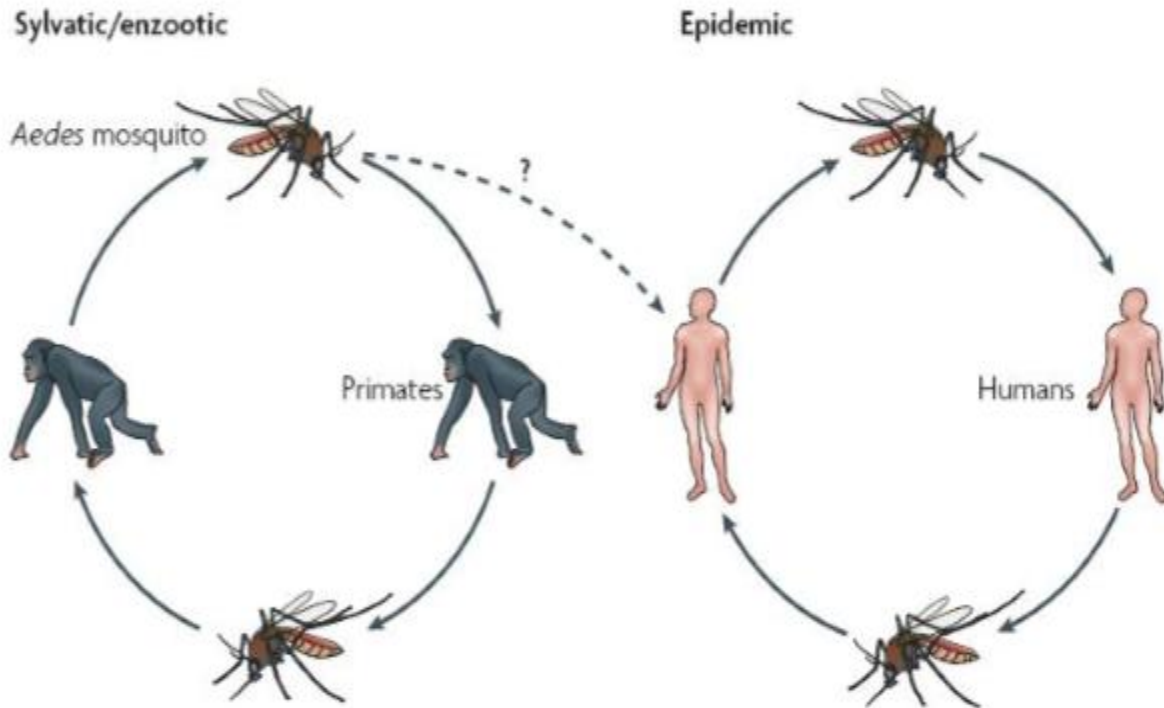


Fig.5 Jumbosep device components (Pall Life Sciences, 2007)

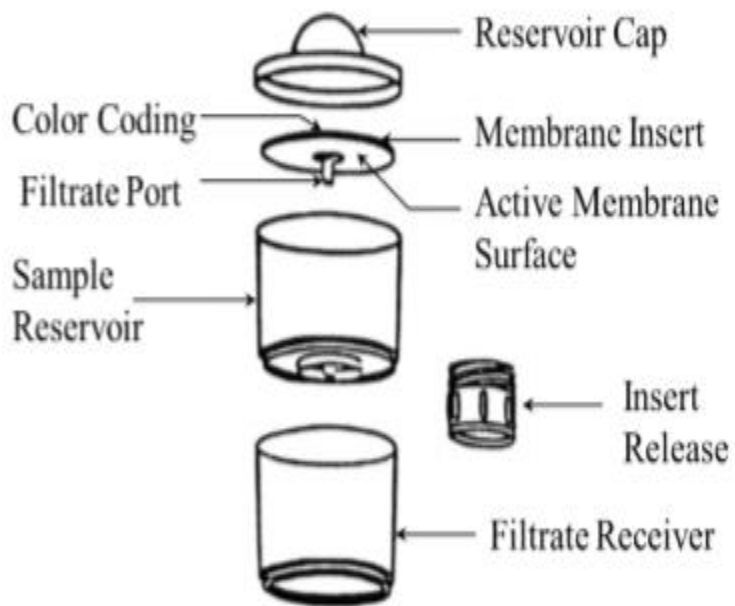


Fig.6 Primary versus secondary DENV infection

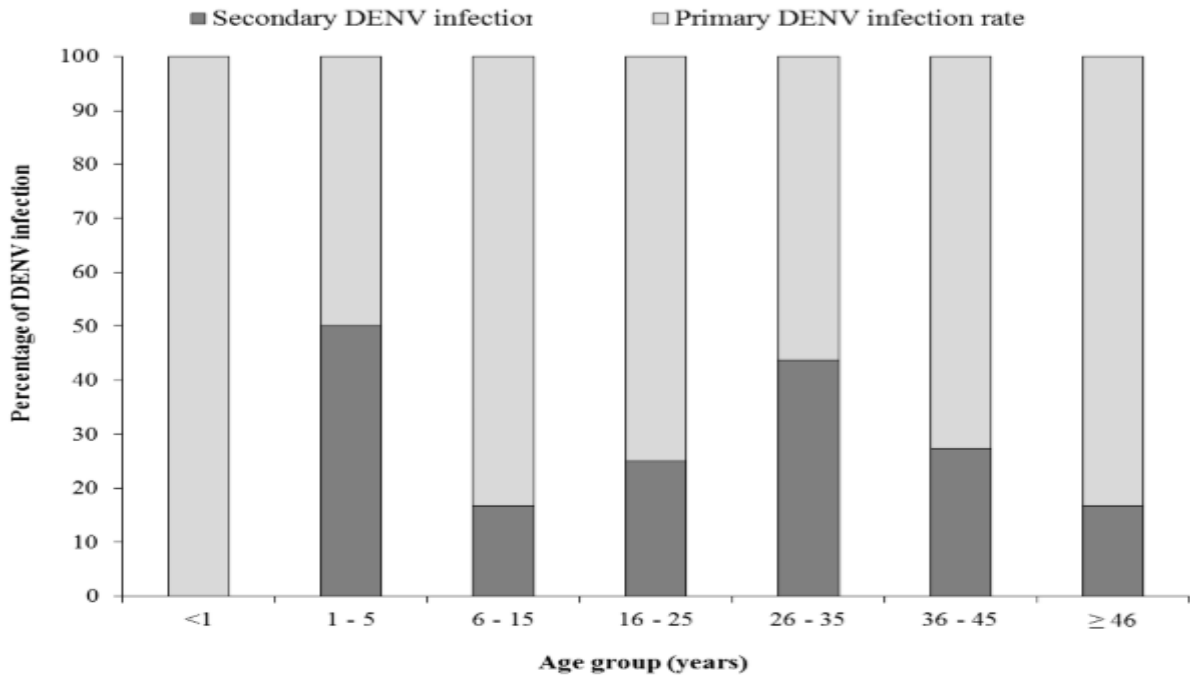


Fig.7 Distribution of DENV infections by age and gender

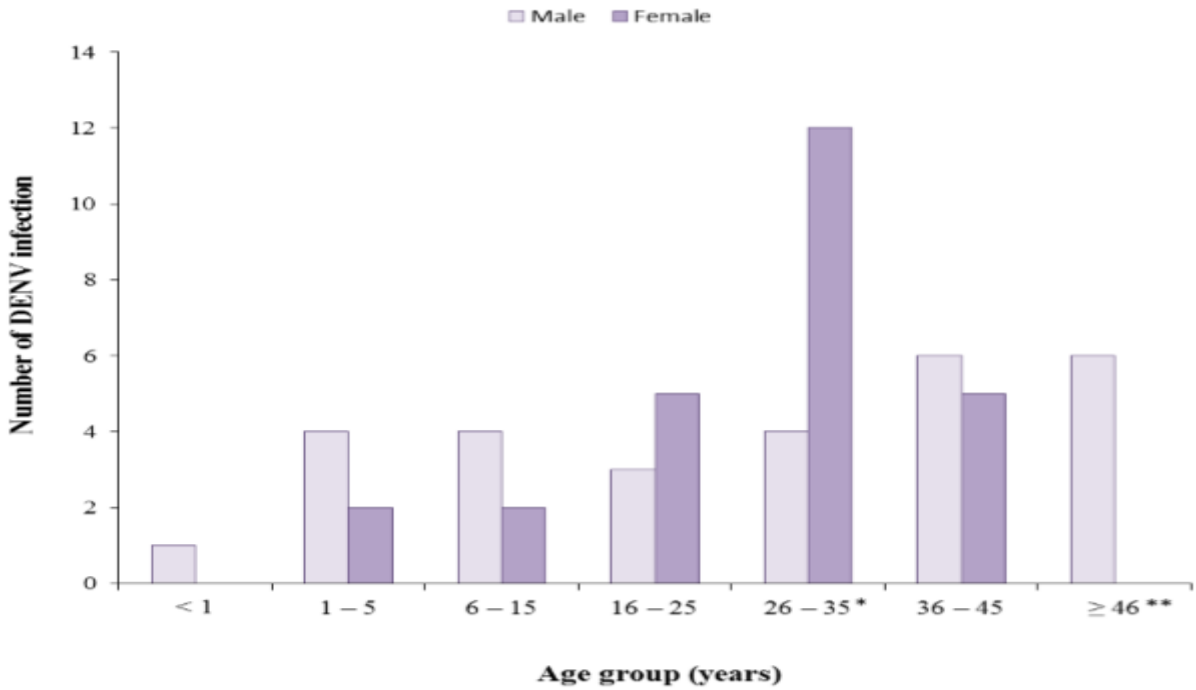


Figure 4.4 Distribution of DENV infections by age and gender.

* p = 0.010

** p = 0.012

The present study concluded that,

- i) A dengue virus infection was one of the causes of acute undifferentiated fever among febrile patients in the county Mombasa.
- ii) Children aged less than 5 years were vulnerable to dengue infection and had a greater risk than adults in developing severe forms of the disease when they acquire a second dengue virus infection with a different serotype.
- iii) Female predominance in dengue cases among age group 26-25 years would have been masked when collapsing the data over all age groups. Therefore, the present study findings indicated the importance of reporting age and gender stratified data for dengue surveillance to help in targeting specific preventive measures.

Recommendations

The present study recommends that:-

- i) The government should provide resources at hospital laboratories to facilitate early diagnosis and management of dengue patients.
- ii) All patients presenting with febrile illness should be tested for dengue antibodies.
- iii) Clinicians/physicians consider the possibility of dengue cases when examining febrile patients.
- iv) The government should initiate dengue surveillance and commence an integrated vector control programme.

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