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

Mustapha Bashir Kazaure*

Department of Science Laboratory Technology, College of Science and Technology, Jigawa State Polytechnic Dutse, Nigeria

*Corresponding author

A B S T R A C T

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 6-15 years (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, Acute fever, Mombasa county

Article Info
Accepted: 07 February 2019
Available Online: 10 March 2019

Introduction
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 6-15 years (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.

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). 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 Ayukkekpong, 2014).

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 (Rothain 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; Coller 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 hematocrit reduction 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 Ayukekpong, 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 our 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 peak 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 been 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 asymptomatically 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 disappear 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°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; Srikiatkhachorn et al., 2007). Some patients remain ill despite normalization of temperature and therefore progresses to DHF. Onset of plasma leakage is characterized by tachycardia and hypotension. The patient sweats, becomes restless, and has 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 hemoconcentration 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,
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 spin 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 sleded 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 tetravalent antigen**

The DENV tetravalent 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 µL/100 µL/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 µl 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 IgG titers of patient sera were determined from a positive standard curve. A sample titer ≥ 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 ≥1:52,000 (Inoue et al., 2010).

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/100µl per well of virus antigen at 4 °C overnight. The wells were blocked with 100µl/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 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/wellof 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 ≥ 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).
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 ≤ 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 sledded 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 Tetravalent Antigen

The DENV tetravalent 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 aliquoted 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 µL/100 µL/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 µl 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 flav 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/100µl per well of virus antigen at 4 °C overnight. The wells were blocked with 100µl/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 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 ≥ 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 ≥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 ≤ 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

| Parameter | Febrile cases n (%) | Dengue cases (IgM +ve) |  |  |  |
|-----------|---------------------|------------------------|---|---|---|
|           |                     | Primary infection IgG titer (<1:52,000) n (%) | Secondary infection IgG titer (≥1:52,000) n (%) | Total dengue cases n (%) | Non-dengue patients (IgM -ve) 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   | 99                  | 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 verses 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.

**References**

Agarwal R, Kapoor S, Nagar R, Misra A, Tandon R and Mathur A. (1999). A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India.

Ahmed S, Arif F, Yahya Y, Rehman A, Abbas K and Ashraf S. (2008). Dengue fever outbreak in Karachi: A study profile and outcome of children under 15 year of age. J Pak Med Assoc. 58:4-8.

Akram DS, Igarashi A and Takasu T. (1998). Dengue virus infection among children with undifferentiated fever in Karachi. Indian J Pediatr. 65: 735-740.

Amarasinghe A, Kuritsk JN, Letson GW and Margolis HS. (2011). Dengue virus infection in Africa. Emerg Infect Dis. 17: 1349–1354.

Anderson KB, Gibbons RV, Edelman R, Eckels KH, Putnak RJ, Innis BL and Sun W. (2011). Interference and facilitation between dengue serotypes in a tetravalent live dengue virus vaccine candidate. J Infect Dis. 204: 442-450.

Anker M and Arima Y. (2011). Male-female differences in the number of reported incident dengue fever cases in six Asian countries. Western Pacific Surveil & Res J. 2(2):17-23.

Amarasinghe A, Kuritsk JN, Letson GW and Margolis HS. (2011). Dengue virus infection in Africa. Emerg Infect Dis. 17: 1349–1354.

Bartlett E, Kotrlik WJ, and Higgins CC. (2001). Organizational research: determining appropriate sample size in survey research. Information technology, learning, and performance. J Spring. 19(1): 43-50.

Bartlet E, Kotrlik WJ, and Higgins CC. (2001). Organizational research: determining appropriate sample size in survey research. Information technology, learning, and performance. J Spring. 19(1): 43-50.

Bartley LM, Donnelly CA, and Garnett GP. (2002). The seasonal pattern of dengue in endemic areas: mathematical models of mechanisms. Trans R Soc Trop Med Hyg. 96: 387-397.

Beatty M, Letson W, Edgil D, and Margolis H. (2007). Estimating the total world population at risk for locally acquired dengue infection. Abstract presented at the 56th Annual Meeting of the Southeast Asian J Trop Med Public Health. 30(4): 735–740.
American Society of Tropical Medicine and Hygiene. Am J Trop Med Hyg. 77(5): 170–257.

Bundo K and Igarashi A. (1985). Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. J. Virol., Methods. 11: 15–22

Bundo K, Morita K, Torres CA, Chanyasanha C, Linn ML, Igarashi A. (1986). Antibody response in Japanese encephalitis and dengue hemorrhagic fever patients measured by indirect ELISA. Trop Med. 28:101–114.

Cardoso IM, Cabidelle AS, Borges PC, Lang CF, Calenti FG, Nogueira, LO, Falqueto A and Junior CC. (2011). Dengue: clinical forms and risk groups in a high incidence city in the southeastern region of Brazil. Revista da Sociedade Brasileira de Med Trop. 44(4):430-435.

CDC. (2010). Dengue and climate. http://www.cdc.gov/dengue/entamology Ecology/climate

CDC. (2012a). Update: Dengue in tropical and subtropical regions. Centers for Disease Control and Prevention, Atlanta.

CDC. (2012b). Laboratory guidance and diagnostic testing. Centers for Disease Control and Prevention, Atlanta.

Chambers TJ, Hahn CS, Galler R and Rice CM. (1990). Flavivirus genome organization, expression, and replication. Annu Rev Microbiol. 44:649–688.

Chaturvedi UC, Agarwal R, Elbishbishi EA and Mustafa AS. (2000). Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol. 28(3):183-188.

Chen LH and Wilson ME. (2004). Transmission of dengue virus without a Mosquito vector, nosocomial mucocutaneous transmission and other routes. Clin Infe Dis. 39:56-60.

Chen L, Ewing D, Subramanian H, Block K, Rayner J, Alterson KD, Sedegah M, Hayes C, Porter K, and Raviprakash K. (2007). A heterologous DNA prime-Venezuelan equine encephalitis virus replicon particle boost dengue vaccine regimen affords complete protection from virus challenge in cynomolgus macaques. J Virol. 81:11634-9.

Cochran WG. (1977). Sampling techniques. 3rd ed. John Wiley & Sons, Inc. New York:

Coller BA and Clements DE. (2011). Dengue vaccines: progress and challenges. Curr Opin Immunol. 23:391-398.

Cologna R and Rico-Hesse R. (2003). American genotype structures decrease dengue virus output from human monocytes and dendritic cells. J Virol. 77: 3929–38.

Crill WD and Roehrig JT. (2001). Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. J Virol, 75:7769-7773.

Durbin AP and Whitehead SS. (2010). Dengue Vaccine Candidates in Development. In Dengue Virus. 338: 129-143.

Dawurung JS, Baba MM, Stephen G, Jonas SC, Bukbuk DN and Dawurung CJ. (2010). Serological evidence of acute dengue virus infection among febrile patients attending Plateau State Specialist Hospital Jos, Nigeria. Report and Opin. 2(6)

Deen JL, Harris E, Wills B, Balmaseda A, Hammond SN, Rocha C, Dung NM, Hung NT, Hien TT, Farrar JJ. (2006). The WHO dengue classification and case definitions: time for a reassessment. Lancet. 368: 170 – 173.

De Wazieres B, Gil H, Vuitton DA and
Dupond JL. (1998). Nosocomial transmission of dengue from a needle stick injury. Lancet. 14:351(910):498

De Souza V. (2007). Sensitivity and specificity of three ELISA based assays for discriminating primary from secondary acute dengue virus infection. J. Clin. Virol. 39:230 – 233.

Duchini A, Govindarajan S, Santucci M, Zampi G and Hofman FM. (1996). Effects of tumor necrosis factor and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS- derived endothelial cells. J Investig Med. 44: 474 – 482.

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

Espina LM, Valero NJ, Hernandez JM and Mosquera JA. (2003). Increased apoptosis and expression of tumor necrosis factor-alpha caused by infection of cultured human monocytes with dengue virus. Am J Trop Med Hyg. 68: 48-53.

Falconar AK. (1997). The dengue virus nonstructural-1 protein (NS1) generates antibodies to common epitopes on human blood clotting, integrin/adhesin proteins and binds to human endothelial cells: potential implications in haemorrhagic fever pathogenesis. Arch Virol. 42(5): 897-916.

Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkhachorn A, Thomas SJ, Srikiatkhachorn A, Yoon IK, Jarman RG, Green S, Rothman AL, Derek A and Cummings T. (2010). Serotype-Specific Differences in the Risk of Dengue Hemorrhagic Fever: An Analysis of Data Collected in Bangkok, Thailand from 1994 to 2006. PLoS Negl Trop Dis. 4(3): 617.

Gerber JS, Mosser DM. (2001). Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. J Immunol.166: 6861–6868

George R and Lum LCS. Clinical spectrum of dengue infection. In: Gubler DJ, Kuno G, eds. (1997). Dengue and dengue hemorrhagic fever. CAB International: New York. pp 89-113.

Gibbons RV and Vaughn DW. (2002). Dengue: an escalating problem. BMJ. 324: 1563 – 1566.

Gibbons RV and Vaughn DW. (2002). Dengue: an escalating problem. BMJ. 324: 1563 – 1566.

Goh KT, Ng SK, Chan YC, Lim SJ and Chua EC. (1987). Epidemiological aspects of an outbreak of dengue fever/dengue haemorrhagic fever in Singapore. Southeast Asian J Trop Med Public Health. 18(3):295–302.

Gubler DJ. (1997). Dengue and dengue haemorrhagic fever: its history and resurgence as a global public health problem. In Dengue and dengue haemorrhagic fever, Edited by Gubler, DJ and Kuno, G. CAB International:Oxford. pp. 1-22.

Gubler DJ, and Clark GG. (1995). Dengue/Dengue Hemorrhagic Fever: The emergence of a global health problem. Emerg Infect Dis. 1: 55-57.

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

Gubler DJ. (2002). Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 10: 100-103.

Gubler DJ and Meltzer M. (1999). Impact of dengue/dengue haemorrhagic fever on the developing world. ADENV Virus Res. 53: 35-70.

Guha-Sapir D and Schimmer B. (2005) Dengue fever: new paradigms for a changing epidemiology. Emerg Themes Epidemiol. 2(1): 1 - 10

Gurugama P, Garg P, Wijewickrama A and Seneviratne SL. (2010). Dengue viral
infections. Indian J Deratol. 55: 68–78.

Guy B, Guirakhoo F, Barban V, Higgs S, Monath TP and Lang J. (2010). Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. Vaccine. 28: 632-649.

Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martinez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S and Peeling RW. (2010). Dengue: a continuing global threat. Nat Rev Microbiol. 8: 7-16.

Guzman MG, Kouri G, Bravo J, Valdes L, and Vazquez S. (2002) Effect of age on outcome of secondary dengue 2 infections. Int J Infect Dis. 6: 118–124.

Guzman MG and Kouri G. (2003). Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. Clin Virol. 27:1-13.

Hales S, De Wet N, Maindonald J and Woodward A. (2002). Potential effect of population and climate changes on global distribution of dengue fever: an empirical model. Lancet 360: 830–834.

Halstead SB. (2002). Dengue. Curr Opin Infect Dis. 15: 471–476.

Halstead SB, Lan NT, Myint TT, Shwe TN, Nisalak A and Kalyanarooj S. (2002). Dengue hemorrhagic fever in infants: Research opportunities ignored. Emerg Infect Dis. 8:1474–1479.

Hammond SN, Balmaseda A, Perez L, Tellez Y, Saborio SI and Mercado JC. (2005). Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. Am J Trop Med Hyg. 73:1063–1070.

Henchal EA and Putnak JR. (1990). The dengue viruses. Clin. Microbiol. Rev. 3: 376-396.

Hertz JT, Munishi OM, Ooi EE, Howe S, Lim WY, Chow A, Morrissey AB, Bartlett JA, Onyango JJ, Maro VP, Kinabo GD, Saganda W, Gubler DJ and Crump JA. (2012). Chikungunya and dengue fever among hospitalized febrile patients in northern Tanzania. Am J Trop Med Hyg. 86(1):171-177.

Huber K, Ba Y, Dia I, Mathiot C, Sall AA, and Diallo M. (2008). Aedes aegypti in Senegal: genetic diversity and genetic structure of domestic and sylvatic populations. Am J Trop Med Hyg. 79: 218-229.

Huismen W, Martina BE, Rimmelzwaan GF, Gruters RA, and Osterhaus AD. (2009). Vaccine-induced enhancement of viral infections. Vaccine. 27: 505-512.

ICTVdB - The Universal Virus Database, version 4. (2006). Virus Taxonomy, Classification and nomenclature of viruses. Columbia University: New York.

Issack MI, Pursem VN, Barkham TMS, Lee-Ching N, and Manraj SS. (2010). Reemergence of Dengue in Mauritius. Emerg Infe Dis. 16(4).

Inoue S, Alonzo MT, Kurosawa Y, Mapua CA, Reyes JD, Dimaano EM, Alera MT, Saito M, Oishi K, Hasebe F, Matias RR, Natividad FF and Morita K. (2010). Evaluation of a dengue IgG indirect enzyme-linked immunosorbent assay and a Japanese encephalitis IgG indirect enzyme-linked immunosorbent assay for diagnosis of secondary dengue virus infection. Vect Born Zoo Dis. 10:143–150.

Jessie K, Fong MY, Devi S, Lam SK and Wong KT. (2004). Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. J Infect Dis 189(8):1411–1418.

Johansson M, Dominici F and Glass G. (2009). Local and Global effect of climate on dengue transmission in
John ALS, Rathore APS, Yap H. (2011). Immune surveillance by mast cells during dengue infection promotes natural killer (NK) and NKT-cell recruitment and viral clearance. Nat Acad Sc. 108(22): 9190–9195.

Johnson BK, Ocheng D, Gichogo A, Okiro M, Libondo D, Kinyanjui P, Tukei PM. (1982). Epidemic dengue fever caused by dengue type 2 virus in Kenya: preliminary results of human virological and serological studies. East Afr Med J. 59: 781–784.

Jury MR. (2008). Climate influence on dengue epidemics in Puerto Rico. Intl J Environ Health Research. 18: 323–334.

Kalayanarooj S, Chansiriwongs V and Nimmannitya S. (2002). Dengue patients at the Children's Hospital, Bangkok: 1995-1999. Review. Dengue Bulletin. 26: 33–43.

Keating J. (2001). An investigation into the cyclical incidence of dengue fever. Soc Sci Med. 53(12): 1587-1597.

Kitchener S, Nissen M, Nasveld P, Forrat R, Yoksan S, Lang J and Saluzzo JF. (2006). mmunogenicity and safety of two live-attenuated tetravalent dengue vaccine formulations in healthy Australian adults. Vaccine. 24: 1238-12341.

Klaassen B. (2010). Dengue/DHF update (23). ProMed. 2010 May 17 [cited 2010 Jun 10]. http://www.promedmail.org, archive no. 20100517.1620

Kenya Integrated House Budget Survey. (2007). Basic report. ISBN: 9966-767-07-X

Kenya National Bureau of Statistics. (2009). The 2009 Kenya Population and Housing Census, volume 1A: population distribution by administrative units.

Khin MM and Than KA. (1983). Transovarial transmission of dengue 2 virus by Aedes aegypti in nature. Am J Trop Med Hyg. 32: 590-594.

Kumar A, Hilaire MG and Nielsen AL. (2013). Epidemiological trends and clinical manifestations of Dengue among children in one of the English-speaking Caribbean countries. Trans R Soc Trop Med Hyg. 10:1093

Leitmeyer KC, Vaughn DW, Watts DM, Salas R, de Chacon VI, Ramos C and Rico-Hesse R. (1999). Dengue virus structural differences that correlate with pathogenesis. J. Virol. 73:4738–4747.

Libraty DH, Endy TP, Houngr HS, Green S, Kalayanarooj S, Sunyayakorn S, Chansiriwongs W, Vaughn DW, Nisalak A and Ennis FA (2002) Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. J Infect Dis. 185:1213–1221

Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, Yang TI, Sceu FC, Kao CF and Lin YS. (2001). Generation of IgM antiplatelet autoantibody in dengue patients. J Med Viol 63:143–149.

Luplerdlop N, Misse D, Bray D, Deleuze V, Gonzalez JP and Leard K. (2006). Dengue-virus- infected dendritic cells trigger vascular leakage through metalloproteinase overproduction. EMBO Rep. 7:1176–1181.

Maves RC, Ore RM, Porter KR and Kochel TJ. (2011). Immunogenicity and protective efficacy of a psoralen-inactivated dengue-1 virus vaccine candidate in Aotus nancymae monkeys. Vaccine. 29: 2691-2696.

Markoff LJ, Innis BL, Houghten R, Henchal LS. (1991). Development of cross-reactive antibodies to plasminogen during the immune response to dengue virus infection. J Infect Dis. 164(2):294-301.

Matlani M and Chakravarti A. (2011).
Changing trends of dengue disease: a brief report from a tertiary care hospital in New Delhi. Braz J Infect Dis. 15: 184-185.

Miller JM, Barend J. M deWet, Luisa Martinez-Pomares, Catherine M Radcliffe, Raymond A Dwek, Pauline M Rudd, Siamon Gordon. (2008). The Mannose Receptor Mediates Dengue Virus Infection of Macrophages PLoS Pathog. 4: e17.

Monath TP. (1994). Dengue: the risk to developed and developing countries. Proc Natl Acad Sci. 91:2395 -2400.

Mongkolsapaya J, Dejnirattisai W, Xu Xn, Vasanawathana S, Tangthawornchaikul N, Chairunsri A, Sawasdivorn S, Duangchinda T, Dong T, Rowland-Jones S, Yenchitsomanus PT, Mcmichael A, Malasit P and Screaton G. (2003). Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nat Med. 9:921-927.

Morrison D, Legg TJ, Billings CW, Forrat, R Yoksan S, Lang J. (2010). A novel tetravalent dengue vaccine is well tolerated and immunogenic against all 4 serotypes in flavivirus naïve adults. J. Infect. Dis. 201(3):370-377.

Mwanganigi JM, Midega J, Kahindi S, Njoroge L, Nzovu J, Githure J, Mbogo CM, and Beier JC. (2011). Mosquito species abundance and diversity in Malindi, Kenya and their potential implication in pathogen transmission. Parasitol Res. 110:61–71

Nakahaporn K and Tripathi NK. (2005). An information value based analysis of physical and climatic factors affecting dengue fever and dengue haemorrhagic fever incidence. Int J Health Geogr. 4: 13.

Nawa M, Takasaki T, Ito M, Inoue S, Morita K, and Kurane I. (2005). Immunoglobulin A Antibody Responses in Dengue Patients: a Useful Marker for Serodiagnosis of Dengue Virus Infection. Clin Diagn Lab Immunol. 12(10): 1235–1237

Ngwe-Tun MM, Thant KZ, Inoue S, Kurosawa Y, Lwin L, Lin S, Aye KT, Khin PT, Myint T, Htwe K, Mapua CA, Natividad FF, Hirayama K, and Morita K. (2013). Serological Characterization of Dengue Virus Infections Observed Among Dengue Hemorrhagic Fever/Dengue Shock Syndrome Cases in Upper Myanmar. Journal of Medical Virology. 9999:1–9

Noble CG, Chen YL, Dong H, Gu F, Lim SP and Schul W. (2010). Strategies for development of dengue virus inhibitors. Antiviral Res. 85(3): 203-209.

Osorio JE, Huang CY, Kinney RM and Stinchcomb DT. (2011). Development of DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever. Vaccine. 29: 7251-7260.

Oishi K, Inoue S, Cinco MTDD, Dimaano EM, Alera MT, Alfon JA, Abanes F, Cruz DJ, Matias RR, Matsuura H, Hasebe F, Tanimura S, Kumatori A, Morita K, Natividad FF, Nagatake T. (2003). Correlation between increased platelet-associated IgG and thrombocytopenia in secondary dengue virus infections. J Med Virol. 71:259–64.

Pang T, Cardosa MJ, Guzman MG. (2007). Of cascades and perfect storms: the immunopathogenesis of dengue haemorrhagic fever-dengue shock syndrome (DHF/DSS). Immunol Cell Biol. 85:43-45.

Perera R, and Kuhn RJ. (2008). Structural proteomics of dengue virus. Annu Rev Microbiol. 11: 369–377.

Perez AB, Sierra B, Garcia G, Aguirre E, Babel N, Alvarez M, Sanchez L, Valdes L, Volk H D, and Guzman MG. (2010)
Tumor necrosis factor-alpha, transforming growth factor-β1, and interleukin-10 gene polymorphisms: implication in protection or susceptibility to dengue hemorrhagic fever. Hum Immunol. 71: 1135–1140.

Peeling RW, Artsob H, Pelegrino JL, Buchy P, Cardosa MJ, Devi S, Enria DA, Farrar J, Gubler DJ, Guzman MG, Halstead SB. (2010). Evaluation of diagnostic tests: Dengue. Nat Rev Microbiol. 8(12): s30-8

Porter KR, Ewing D, Chen L, Wu SJ, Hayes CG, Ferrari M, Teneza-Mora N, and Raviprakash K. (2012). Immunogenicity and protective efficacy of a vaxfectin-adjuvanted tetravalent dengue DNA vaccine. Vaccine. 30:336-341.

Pun R, Pant KP, Bhatta DR and Pandey BD. (2011). Acute Dengue Infection in the Western Terai Region of Nepal. J Nepal Med Assoc. 51(181): 11-14

Reiter P. (2001). Climate change and mosquito-borne disease. Environ Health Perspect. 109(1): 141-61.

Rigau-Perez JG. (2006). Severe dengue: the need for new case definitions. Lancet Infect Dis. 6:297-302.

Roiz D, Eritja R, Molina R, Melero-Alcibar R and Lucientes J. (2008) Initial distribution assessment of *Aedes albopictus* (Diptera: Culicidae) in the Barcelona, Spain, Area. J Med Entomol. 45: 347-352.

Rothman AL. (2004). Dengue: defining protective versus pathologic immunity. J Clin Invest. 113: 946-951.

Rothman AL. (2011). Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol. 11: 532-543.

Rodhain F and Rosen L. (1997). Mosquito vectors and dengue virus-vector relationships. In: Gubler DJ, Kuno G. Dengue and Dengu Hemorrhagic Fever. CAB International, New York: USA. 45 - 60.

Rothman AL. (1999). Viral pathogenesis of dengue infections. In Anonymous Dengue and dengue hemorrhagic fever. CABI Publishing, New York.

Saito M, Oishi K, Inoue S, Dimaano EM, Alera MTP, Robles MP, Estrella JR, Kumatori A, Moji K, Alonzo BMT, Buerano CC, Matias RR, Morita K, Natividad FF, Nagatake T. (2004). Association of increased platelet-associated immunoglobulins with thrombocytopenia and the severity of disease in secondary dengue virus infections. Clin Exp Immunol 138: 299–303. Sang R and Dunster LM. (2001). The growing threat of arbovirus transmission and outbreaks in Kenya: a review. East Afr Med J. 78(12):655-661.

Sante-Plus.Org. (2010). [in French, trans. ProMed MOD.TY, edited] <http://sante-plus.org/breve663.html>

Sanchez V, Gimenez S, Tomlinson B, Chan PK, Thomas G, Forrat R, Chambonneau L., Deauvieau F, Lang J and Guy B. (2006). Innate and adaptive cellular immunity in flavivirus- naive human recipients of a live-attenuated dengue serotype 3 vaccines produced in Vero cells (VDV3). Vaccine. 24: 4914-4926.

Sharma Y, Kaur M, Singh S, Pant L, Kudesia M, Jain S. (2012). Seroprevalence and trend of dengue cases admitted to a government hospital, Delhi – 5-year study (2006-2010): A look into the age shift. Int J Prev Med. 3:537-43.

Shrestha B, Brien JD, Sukupolvi-Petty S, Austin SK, Edeling MA and Kim T. (2010). The development of therapeuic antibodies that neutralize homologus and heterologous genotypes of dengue virus type 1. PLoS Pathog. 6(4): e1000823.

Siddiqui FJ, Haider SR, and Bhutta ZA.
Endemic Dengue Fever: a seldom recognized hazard for Pakistani children. J Infect Dev Ctries. 3(4): 306-312

Soundravally R and Hoti SL. (2007). Immunopathogenesis of dengue hemorrhagic fever and shock syndrome: role of TAP and HPA gene polymorphism. Hum Immunol. 68: 973

Srikiatkhachorn A, Krautrachue A, Ratanaprakarn W, Wongtapradit L, Nithipanya N and Kalayanarooj S. (2007). Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. Pediatr Infect Dis J. 26:283–290.

Sissoko D, Ezzedine K, Giry C, Moendandze A, Lernout T, D’Ortenzio E, Pettinelli F, Malvy D. (2010) Seroepidemiology of Dengue Virus in Mayotte, Indian Ocean, 2006. PLoS ONE 5(11): 14141.

Stephens HA. 2010. HLA and other gene associations with dengue disease severity. Curr Top Microbiol Immunol. 338: 99-114.

Stephenson JR. (2005). Understanding dengue pathogenesis: implications for vaccine design. Bull World Health Organ 83:308–314.

Tan FL, Loh DL, Prabhakaran K, Tambyah PA and Yap HK. (2005). Dengue haemorrhagic fever after living donor renal transplantation. Nephrol Dial Transplant. 20:447–448.

Tank AG, Jain MR. (2012). Trend of dengue in a tertiary care hospital of surat city, western India. Nat J Comm Med. 3(2): 302-304

Tassaneentitheap B, Burgess TH, Granelli-Piperno A, Trumpfheller C, Finke J, Sun W, Eller MA, Pattanapanyasat K, Sarasombath S, Bixr DL, Steinman RM, Schlesinger S and Marovich MA. (2003). DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med. 197(7): 823-829

Thomas SJ. (2011). The necessity and quandaries of dengue vaccine development. J Infect Dis, 203: 299-303.

Tomashek KM, Rivera A, Muñoz-Jordan JL, Hunsperger E, Santiago L, Padro O, Garcia E and Wellington S. (2009). Description of a large island-wide outbreak of dengue in Puerto Rico, 2007. 81:467–474.

Ty Hang VT, Holmes EC, Veasna D, Quy NT and Tinh Hien T. (2010). Emergence of the Asian 1 Genotype of Dengue Virus Serotype 2 in Viet Nam: In Vivo Fitness Advantage and Lineage Replacement in South-East Asia. PLoS Negl Trop Dis. 4(7): 757.

Vairo F, Nicastri E, Meschi S, Schepisi MS, Paglia MG, Bevilacqua N, Mangi S, Sciarrone MR, Chiappini R, Mohamed J, Racalbuto V, Di Caro A, Capobianchi MR and Ippolito G. (2012). Seroprevalence of dengue infection: a cross-sectional survey in mainland Tanzania and on Pemba Island, Zanzibar. Int J Infect Dis. 16(1):44-46.

Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Endy TP, Raengsakulrach B, Rothman AL, Ennis FA and Nisalak A. (2000). Dengue viraemia titre, antibody response pattern, and virus serotype correlate with disease severity. J Infect Dis. 181: 2

Voller A, Bidwell O, and Bartlett A. (1976). Microplate enzyme immunoassays for the immunodiagnosis of viral infections. 506 – 512. In NR Rose and Friedman (ed). Manual of Clinical Immunology, Amer Soc Microbiol, Washington D.C.

Whitehead SS, Blaney JE, Durbin AP and Murphy BR. (2007). Prospects for a dengue virus vaccine. Nat Rev Microbiol. 5: 518-528.

WHO. (1997). Dengue Haemorrhagic Fever:
Diagnosis, Treatment, Prevention and Control. 2nd edition. WHO. Geneva, Switzerland

WHO. (2007a) Scientific Working Group Report on Dengue. WHO. Geneva, Switzerland.

WHO. (2007b). Addressing sex and gender in epidemic-prone infectious diseases. WHO. Geneva, Switzerland.

WHO. (2009). Dengue: guidelines for diagnosis, treatment, prevention and control. WHO. Geneva, Switzerland.

WHO. (2011). Somalia Emergency Health Update. Weekly Highlights 19-25 November 2011

WHO. (2012). Report on the Subregional meeting on dengue fever in the Red Sea rim. WHO. Regional Office for the Eastern Mediterranean.

Wilder-Smith A, Chen LH, Massad E and Wilson ME. (2009). Threat of dengue to blood safety in dengue-endemic countries. Emerg Infect Dis.

Wongkoon S, Jaroensutasinee M, Jaroensutasinee K and Preechaporn W. (2007). Development sites of Aedes aegypti and Ae. albopictus in Nakhon Si Thammarat, Thailand. Dengue Bull. 31: 141-152.

Wu SJ, Grouard-Vogel G, Sun W, Mascola JR, Brachet E, Putvatana R, Louder MK, Filgueira L, Marovich MA, Wong HK, Blauvelt A, Murphy GS, Robb ML, Innes BL, Birx DL, Hayes CG and Frankel SS. (2000). Human skin Langerhans cells are targets of dengue virus infection. Nat Med. 6(7):816-20.

Yang HM, Macoris MLG, Galvani KC, Andrighetti MTM and Wanderley DMV. (2009). Assessing the effects of temperature on the population of Aedes aegypti, the vector of dengue. Epidemiol Infect. 137:1188-202.

Yauch LE and Shresta S. (2008). Mouse models of dengue virus infection and disease. Antiviral Res. 80(2): 87-93.

Yauch LE, Zellweger RM, Kotturi MF, Qutubuddin A, Sidney J, Peters B, Prestwood TR, Sette A and Shresta S. (2009). A protective role for dengue virus-specific CD8+ T cells. J Immunol. 182(8):4865-73.

Yen TY, Chen HC, Lin YD, Shieh CC, and Wu-Hsieh BA. (2009). Enhancement by Tumor Necrosis Factor Alpha of Dengue Virus-Induced Endothelial Cell Production of Reactive Nitrogen and Oxygen Species Is Key to Hemorrhage Development. J Virol. 83:18

Yew Y, Ye T, Ang L, Ng L, Yap G, A and James L. (2009). Seroepidemiology of dengue virus infection among adults in Singapore. Annual Acad Med. 38: 667-675.

Zanotto PM, Gould EA, Gao GF, Harvey PH and Holmes EC. (1996). Population dynamics of flaviviruses revealed by molecular phylogenies. Proc Natl Acad Sci. 93:548–553.

How to cite this article:

Mustapha Bashir Kazaure. 2019. Analysis of Dengue Fever among Patients Attending Dutse General Hospital in Jigawa State, Nigeria. Int.J.Curr.Microbiol.App.Sci. 8(03): 485-514.
doi: https://doi.org/10.20546/ijcmas.2019.803.061