PREVALENCE OF DENGUE FEVER AND INVESTIGATION OF INCIDENCE OF DENGUE HEMORRHAGIC FEVER AND DENGUE SHOCK SYNDROME AMONG PATIENTS ATTENDING THE COAST PROVINCE GENERAL HOSPITAL

BY

NANCY NZALAMBI KUTTOH

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DECLARATION

I declare that thesis is my original work and has not been presented for a degree or other awards in any other university.

Signature:……. Date:…….

Nancy Nzalambi Kuttoh

EL/ESM/00596/2013

This thesis has been submitted for examination with our approval as the supervisors.

Signature:…… Date……..

Dr. Guyah Bernard

School of Public Health and Community Development, Maseno University

Signature:…… Date:……

Dr. Shingo Inoue

Nagasaki University/ Kenya Medical Research Institute, Production Department

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I dedicate this thesis to my son Raymond: my parents, Chirongo and Mujeni and my siblings Dena, Kuttoh, Zidi and Gereza. You all have been my source of inspiration and encouragement.

ABSTRACT

Dengue is the most rapidly spreading arbovirus infection globally and exhibits a substantial temporal and geographical variability. The number of dengue cases reported annually to WHO has progressively increased from 0.4 to 1.3 million in the decade 1996–2005, reaching 2.2 million in 2010 and 3.2 million in 2015. Kenya has experienced heightened cases of dengue in recent years with outbreaks in Mandera in 2011 and Mombasa in 2012, 2013 and 2017. Prevalence of dengue among febrile patients in Mombasa in 2015 was 15% as the latest published data show. Dengue virus (DENV) has four closely related but serologically distinct viruses denoted as DENV-1, DENV-2, DENV-3, and DENV-4. Clinical manifestation of dengue ranges from mild-self resolving dengue fever (DF) to severe life threatening forms of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Major risk factors for DHF and DSS are mosquito vector population and secondary infection of DENV 1- 4. With no direct treatment for dengue, patients are normally given supportive therapy to aid healing. The progression to DHF and DSS occurs shortly after the fever has subsided and this may mislead health care providers that the patient is leading to recovery. The burden of DHF and DSS is poorly quantified with increasing geographic expansion to new countries. The study was aimed at determining the prevalence of DF, primary and secondary infections and investugating the incidence of DHF and DSS. This was a hospital-based cross-sectional study where 203 patients attending the Coast Province General Hospital and presenting with fever were recruited between October 2016 and May 2017. The DENV specific IgM and IgG antibodies were determined using enzyme-linked immunosorbent assay (ELISA). Results showed that 36 (17.7%) out of 203 febrile cases were positive for anti-DENV IgM. About twenty one cases (10.3%) were primary infections and 15 (7.4%) were secondary infections. None of the patients could fit the criteria for DHF/DSS. The relationship between age groups and type of infection, whether primary or secondary, was found to be significant (p=0.027) with primary infections highest (4.5%) in the 30-44 age groups whereas secondary infections were highest (4%) in age group 15-29. No incidence of DHF or DSS was reported in this study. However, study reported a prevalence increase from previous years showing the endemic nature of dengue. Adequate public health planning to avert the life threatening DHF and DSS is required since dengue is a rapidly increasing global public health threat.

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ABBREVIATIONS AND ACRONYMS

Ab : Antibody

Ag : Antigen

ADE : Antibody Dependent Enhancement

ALT : Alanine aminotranferase

AST : Aspartate amino transferase

CPGH : Coast Province General Hospital

CBC : Complete Blood Count

DC : Dendritic cells

DENV : Dengue Virus

DF : Dengue Fever

DHF : Dengue Hemorrhagic Fever

DSS : Dengue Shock Syndrome

ELISA : Enzyme Linked Immunosobent Assay

ICTV : International Committee on Taxonomy of Viruses

IgG : Immunoglobulin G

IgM : Immuniglobulin M

JEV : Japanese Encephalitis Virus

KEMRI : Kenya Medical Research Institute

NK : Natural Killer cells

NS : Non-Structural

OD : Optical Density

OPD : *o*-Phenylenediamine Dihydrochloride

PD : Production Department

rpm : revolutions per minute

SPSS : Statistical Package for the Social Sciences

WHO : World Health Organization

ZDU : Zoonotic Disease Unit

DEFINITION OF TERMS

Serotype – A distinct variation within a species of dengue virus (DENV)

Primary infection – First infection by any DENV serotype

Homotypic re-infection – Second infection by the same DENV serotype

Secondary infection – Re-infection by a different DENV serotype

Antibody Dependent Enhancement - when non-neutralizing antibodies facilitate virus entry into host cells, leading to increased infectivity in the cells

Thrombocytopenia – Decreased platelet level

Leucopenia – Decreased white blood cells level

Hemoconcentration – Decreased plasma volume with simultaneous increase in accumulation red blood cells and other constituents of blood

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Dengue is a mosquito-borne flavivirus infection found in the tropical and subtropical regions around the world that has in the recent years caused international public health concern. (Oishi, et.al., 2007). Dengue exhibits substantial temporal and geographic variability (Limkittikul et \al., 2014) Transmission intensity as well as population structure and demographic factors such as residence, occupation, age and marital status can affect distribution of dengue virus infections and cases (WHO, 2016).

The number of dengue cases reported annually to WHO has increased from 0.4 to 1.3 million in the decade 1996–2005, reaching 2.2 million in 2010 and 3.2 million in 2015 (WHO, 2016). Instances of dengue occurring in Africa from 1948, data is based on published reports of outbreaks and serosurveys, and reports of dengue diagnosis in suspected cases of travelers returning from Africa (WHO, 2009). The first outbreak of dengue fever (DF) in Kenya occurred in 1982 in the coastal towns of Malindi and Kilifi (Chepkorir, 2014). DF is currently re-emerging in Mombasa causing major outbreaks. In 2012, a prevalence of 13.9% was reported (Omuyundo, 2014) which appeared to be higher as compared to study findings from the neighbouring country (Tanzania) that reported 4.5% and 9.5% of dengue cases among the febrile patients (Vairo *et al*., 2012; Hertz *et al*., 2012). A study done by Munyuga and her colleagues (2014, 2015) shows that prevalence in Mombasa continues to be in the rise (15%). Dengue has now become endemic in Mombasa and surveillance is therefore of paramount importance to monitor disease burden and pattern of occurrence.

Dengue virus (DENV) infections of humans were long thought to be self-limited and of low mortality (Halstead, 2015). The transmission cycle of DENV involves the *Aedes* mosquito and lower primates in the rain forests of Asia and Africa (Guble, 1998). A number of *Aedes* mosquitoes, such as *Ae. Aegypti* and *Ae. Albopictus*, may act as a vector in these situations. The most important transmission cycle is the urban epidemic cycle in tropical and subtropical countries. DENV is now the most frequent cause of arboviral diseases world-wide, and it has become a major public health concern, particularly in these areas (Oishi et al., 2007). Environmental temperatures and vector competence to transmit DENV explain the ready transmission in Mombasa where mean temperatures are generally high and the vectors are circulating in the region (Chepkorir, 2014).

Symptomatic cases of DENV infections are classified into dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The occurrence of dengue outbreaks is linked to a number of factors, including the density of mosquitoes during rainy season, particularly that of *Aedes aegypti* (WHO, 1997) and severe disease occurs during a second heterotypic DENV infection or during a first DENV infection in infants born to dengue-immune mothers. (Halstead, 2015).

Mild type of dengue which is DF is clinically defined as an acute, flu-like illness that affects infants, young children and adults, but seldom causes death. DF shows high fever above 38.5 °C accompanied by the following symptoms: severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands and/or rash. Symptoms usually last for 5–10 days, after an incubation period of 4–10 days after the bite from an infected mosquito (Omuyundo, 2014). Laboratory confirmation of DF is basically done by detection of anti-DENV IgM and IgG

antibodies (WHO, 1997). Due to lack of direct treatment, only supportive care based on careful monitoring of patient's vital conditions and laboratory confirmation could help their recovery (Omuyundo, 2014). The severe dengue (DHF/DSS) manifestations that develop in a small proportion of dengue-infected patients occur relatively late in the course of the illness, usually day 4–6, at the time of fever clearance (Halstead, 2015). DHF and DSS have potentially deadly complications due to plasma leakage, respiratory distress, severe bleeding, or organ impairment. Warning signs occur in the late acute phase (Sellahewa, 2013). Such cases can be tested for complete blood count and liver enzyme levels where increase in hematocrit levels (hemoconcentration) and rapid decrease in platelet levels (thrombocytopenia) are considered as warning signs. The patient may need to be hospitalized and given supportive care and management to avert the progression to DHF and DSS (WHO, 2012; Gupta, 2014).

Clinical manifestations for DHF include severe abdominal pain, fatigue, restlessness, rapid breathing, persistent vomiting, and epistaxis, bleeding gums, melena and hematemesis. The next 24–48 hours of the critical stage can be lethal; proper medical care is needed to avoid complications and risk of death (Sellahewa, 2013). Plasma leakage is specific to the pleural and abdomen. In DHF there is no vasculitis and hence no injury to the vessel walls, and plasma leakage results from cytokine mediated increase in vascular permeability (Sellahewa, 2013). DSS includes all bleeding tendencies and in addition circulatory failure due to blood loss and hemoconcentration. The skin of the patient becomes cold and blotchy and further more patients show weak pulse, low blood pressure, and profound shock and eventually die. Since there is no direct medication for dengue, it is recommended that patients have sufficient rest and supportive treatment including IV fluids and analgesic drugs given. Aspirin (Acetylsalicylic acid) or

ibuprofen is contraindicated in dengue patients since they can increase the risk of bleeding (WHO, 2009).Multivitamins may be prescribed where the patient shows low appetite.

DENV has 4 closely related but serologically distinct serotypes denoted as DENV-1, DENV-2, DENV- 3 and DENV- 4. Thus persons living in dengue endemic areas with multiple serotypes of DENV can be infected twice, thrice or probably four times during their lifetime(Gubler, 1998).

Infection by one of the four DENV serotypes (primary infection) has been shown to confer lasting protection against future infection by that serotype (homotypic re-infection). However, re-infection by a different serotype (secondary heterotypic infection) is associated with an increased risk of DHF and DSS. This and other observations suggest an immunopathological component in dengue pathogenesis, which is referred to as antibody dependent enhancement of a disease (Omuyundo,2014) when non-neutralizing antiviral proteins e.g antibodies facilitate virus entry into host cells, leading to increased infectivity in the cells, increased viremia and a more severe set of symptoms. Determination of primary and secondary infections in dengue confirmed cases is a vital indicator to monitor DHF and DSS.

Since DF cases have already been reported in Mombasa, with no information of DHF or DSS, this study went further from the previous prevalence studies to investigate the incidence of DHF and DSS among the population seeking medical attention at the Coast Province General Hospital (CPGH) between the months of October 2016 and May 2017. Clinical manifestations as well as sound laboratory testing including serology and complete blood count tests were used to classify dengue cases. DF cases that showed warning signs for DHF and DSS were admitted and observed on daily basis. The study duration (October 2016 to May 2017) was considered as the season of short and long rains which are attributed to increased mosquito vector breeding.

1.2. Statement of the Problem

Dengue fever clinical symptoms are difficult to distinguish from other common febrile illnesses such as malaria. Available evidence so far indicates that DENV -1, -2 and -3 appear to be a common cause of acute fever in Kenya, Sudan and Somalia in Eastern Africa. The existence of several DENV serotypes in one geographical region exposes the population to secondary infections that increases the risk of developing severe dengue infections DHF and DSS. Despite presence of the mosquito vector responsible for transmission and past studies showing evidence of DENV-1, -2, -3 and -4 circulating in the region, no case of DHF or DSS has been reported as these studies did not go further to investigate DHF and DSS. Lack of detailed epidemiological data based on DHF and DSS is a major gap in monitoring the trend of dengue in Mombasa where epidemiological data show has the highest burden of the disease in Kenya and previous studies have only concentrated on the general prevalence of the disease.

1.3 General Objective

To investigate the prevalence of DF and investigate the incidence of DHF and DSS among the patients attending the Coast Province General Hospital between October 2016 and May 2017.

1.3.1 Specific Objectives

1. To screen and confirm cases of DF in febrile patients attending CPGH between October 2016 and April 2017 to determine the prevalence.

2. To find out the percentage of primary and secondary infections among using IgM capture ELISA and indirect IgG ELISA.

3. To determine the incidence of DHF and DSS using clinical manifestations, complete blood count test, chest X-ray and abdominal ultrasound.

1.4 Research Questions

1. What is the prevalence of DF cases among febrile patients attending CPGH between October 2016 and May 2017?

2. What is the percentage of primary as well as secondary infections among dengue confirmed cases?

3. What is the incidence of DHF and DSS among the dengue confirmed cases Coast County Hospital?

1.5 Justification

The main public health impact of dengue occurs during outbreaks of this disease. Similarity of clinical manifestations with other febrile illnesses such as malaria make early stages of an outbreak grossly under-reported until the epidemic is recognized as dengue, which is usually near peak transmission; it then becomes grossly over reported. With the endemic nature of dengue at the Kenyan coast, it can be perceived that secondary infections could occur in patients because of the four serotypes of DENV. Secondary infections have been linked to be one of the main reasons for severe dengue in patients that result in life threatening conditions such as DHF and DSS. Since DF cases have already been reported in Mombasa, with no information of DHF or DSS, this study explored the incidence of DHF and DSS among the population seeking medical attention at the Coast Province General Hospital (CPGH) between the months of October 2016 and May 2017. The duration was considered as the season of short and long rains which are attributed to increased mosquito vector breeding.

1.6 Study Significance

The study findings are intended to help the physicians to consider possibility of dengue cases when handling febrile patients resulting in proper management of dengue patients to avoid fatal complications. Additionally, the study gives information that will help government and non-governmental partners and the community to implement policies on adequate infection prevention practices and improve vector control programmes to reduce the dengue burden thus benefiting the community at large. The findings also form a base for further research in the epidemiology and molecular characteristics of endemic strains in efforts to prevent and control occurrences of severe

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology

Dengue is one of the fastest spreading mosquito-borne disease in the world and is endemic in most tropical and sub-tropical countries with an estimated 96 million infections resulting in clinical disease annually (Kosasih *et al* 2016). . The World Health Organization (WHO) estimates that 50–100 million DENV infections occur each year and that almost half the world's population lives in countries where dengue is endemic.

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. Clinical presentation in both children and adults may vary in severity depending on the immune status.

Figure 2.1.1: World Distribution of Dengue (WHO, 2014)

Beginning in the 1950s, at the time when four different DENVs were discovered, a lethal variant of dengue emerged. DHF/DSS initially observed in Southeast Asia now has spread throughout the world (Halstead, 2015)

Before the 1980s, little was known of the distribution of DENV in Africa. Instances of dengue occurring in Africa from 1948, data is based on published reports of outbreaks and serosurveys, and reports of dengue diagnosis in suspected cases of travelers returning from Africa. (WHO, 2009). These reports indicate there was a substantial increase in epidemic dengue activity in Africa during the 1980s. Since then, however, major epidemics caused by all four serotypes have occurred in both East and West Africa (Gubler 1994; 1997). Outbreaks have been more common in East Africa and the Middle East in the 1990s, with major epidemics in Djibouti in 1991 caused by DENV-2 virus and in Jeddah, Saudi Arabia, in 1994; both were the first outbreaks in those countries in over 50 years (Gubler, 1997; Rodier, 1995). It is reported that currently close to 75% of the global population exposed to dengue are in the Asia- Pacific region. (WHO, 2012).

Nearly 300,000 cases were reported in 5 large epidemics in the Seychelles (1977–1979), Réunion Island (1977–1978), Djibouti (1992–1993), Comoros (1992–1993), and Cape Verde (2009) (Amarasinghe, Kuritsk, Letson, & Margolis, 2011). Available evidence so far indicates that DENV-1, -2 and -3 appear to be a common cause of acute fever in Kenya, Sudan and Somalia in eastern Africa (Sang, 2005). The 1992-1993 dengue out break reported among United States troops engaged in a mission in Somalia, which lies to the north of Kenya, showed that 41% of cases were caused by DENV-2 while 2% were caused by DENV-3 (Sharp, DeFraites, Thornton, Burans, & Wallace, 1995).

A cross-sectional survey of febrile outpatients performed in 2007 in mainland Tanzania, a sporadic transmission of DENV infection with a 4.5% seroprevalence rate was detected. A significantly higher seroprevalence was reported among adult patients from Pemba Island reaching a rate of 15.4%. (Vairo *et al*., 2012).

A more recent dengue epidemic study that affected different regions of Sudan in 2010, it was reported that 5 out of 113 patients died due to the disease; 3 of them had DHF and the other 2 had DSS (Abdallah, 2012). With no effective vaccine yet, no public health planning and no properly implemented emergency response plan, it is possible severe type of dengue is spreading and causing mortalities across neighbouring countries which are considered dengue endemic. Kenya is considered as a dengue endemic country by the WHO. Active surveillance in Kenya in 2011 confirmed cases of dengue in Mandera, Daadab and Nairobi. In a study done in Mombasa in 2012, it is reported that DF had a prevalence of 13.9%.

The virus continues to be present even after the outbreak that occurred in 2012. A study by Munyuga *et al.*, (2014, 2015) shows that DENV 2 was the predominant serotype and that prevalence continues to be in the rise (15%). It is evident from this study that DENV serotypes 1-4 are co-circulating at the Kenyan Coast and the patients who are co infected with two or more of the serotypes are more as compared to those infected with a single serotype. The co-circulation of dengue virus serotypes suggests that dengue virus is endemic at the Kenyan coast. Even with this there is still no sign of DSS and DHF in the study area. Therefore, there is need to monitor trends and disease burden of dengue and investigate for possible DHF and DSS in the area since cases of dengue continue to be on the rise in countries that report to WHO as shown in Figure 2.2.

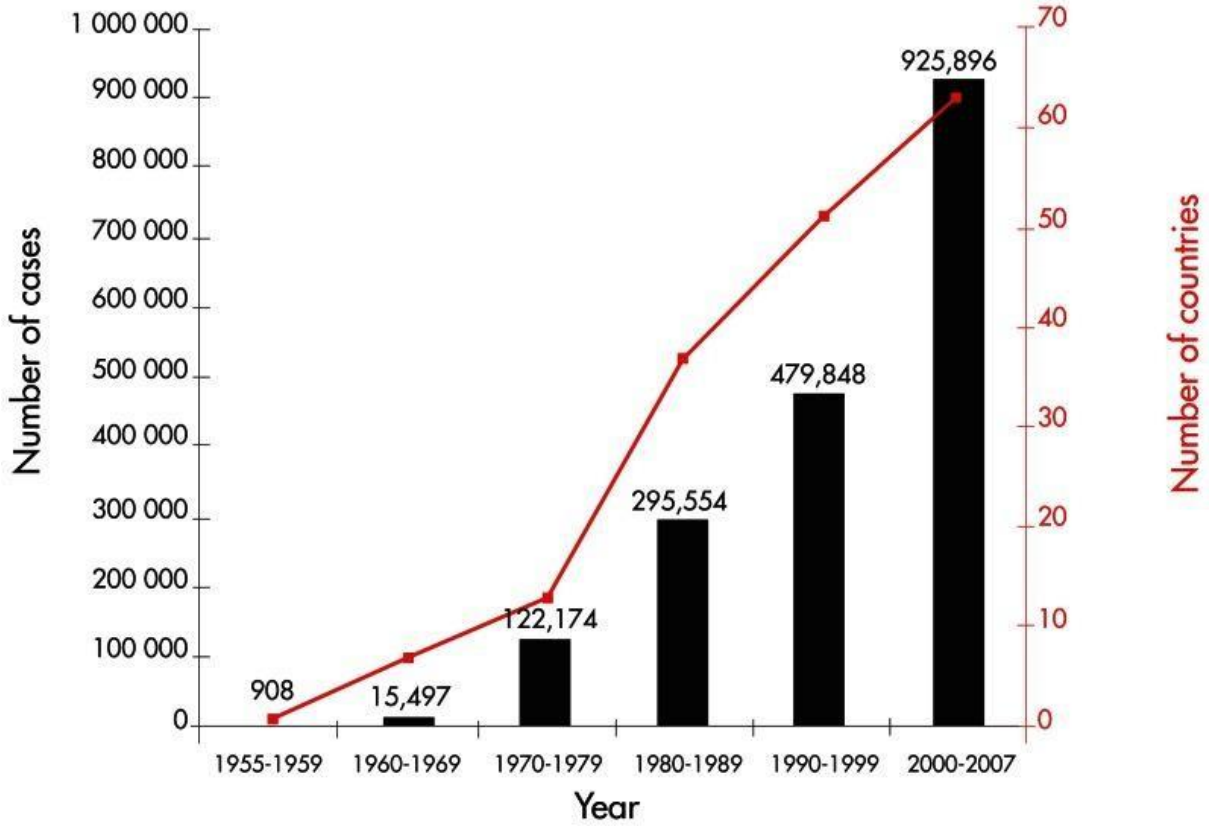


Figure 2.1.2.Average annual number of dengue fever (DF) and dengue haemorrhagic fever (DHF) cases reported to WHO, and of countries reporting dengue, 1955–2007 (WHO,2009)

Since epidemic dengue activity has mostly been classical DF without associated mortality in Kenya, dengue has not been seen as a disease that should be given priority when compared with malaria and HIV and AIDS. Due to this, dengue surveillance data is sparse with the only published prevalence data done in 2012 and 2015. It is important therefore to track the trend of dengue in Mombasa to provide data on annual prevalence rates and proportions of primary and secondary infections to monitor any changes in the trend. With evidence showing 4 serotypes of DENV circulating in Mombasa, data on monitoring the emergence of DHF and DSS will be

important to answer the big scientific question of whether DHF and DSS cases exist in Mombasa and for prevention of these life threatening types of dengue since there is no vaccine yet nor direct treatment for dengue.

2.2 Dengue Virus Structure

DENV is a positive-sense, single-stranded RNA enveloped virus that belongs to family *Flaviviridae* and genus *Flavivirus* (ICTVdB, 2006). the flaviviruses are relatively small, 40-50nm and spherical with lipid bilayer envelope as shown in Figure 2.3.

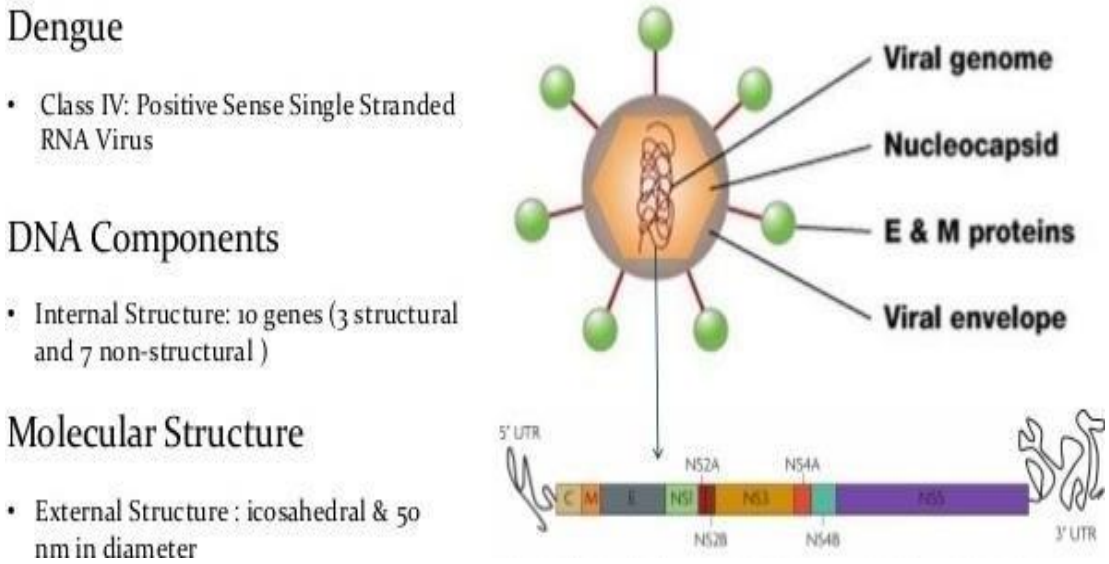


Figure 2.2.1: Dengue virus (Kuhn, *et al*., 2002)

DEV genome is about 11000 bases of positive-sense single stranded RNA (ssRNA) that codes for three that codes for three structural proteins (capsid protein C, membrane rotein M, envelope protein E) and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) as illustrated in Figure 2.4 below (Gubler, 1998). They have common epitopes on the envelope protein that result in extensive cross-reactions in serologic tests. These make

unequivocal serological diagnosis of flavivirus difficult, which is especially true among the four dengue viruses (Zanotto *et al.*, 1996).

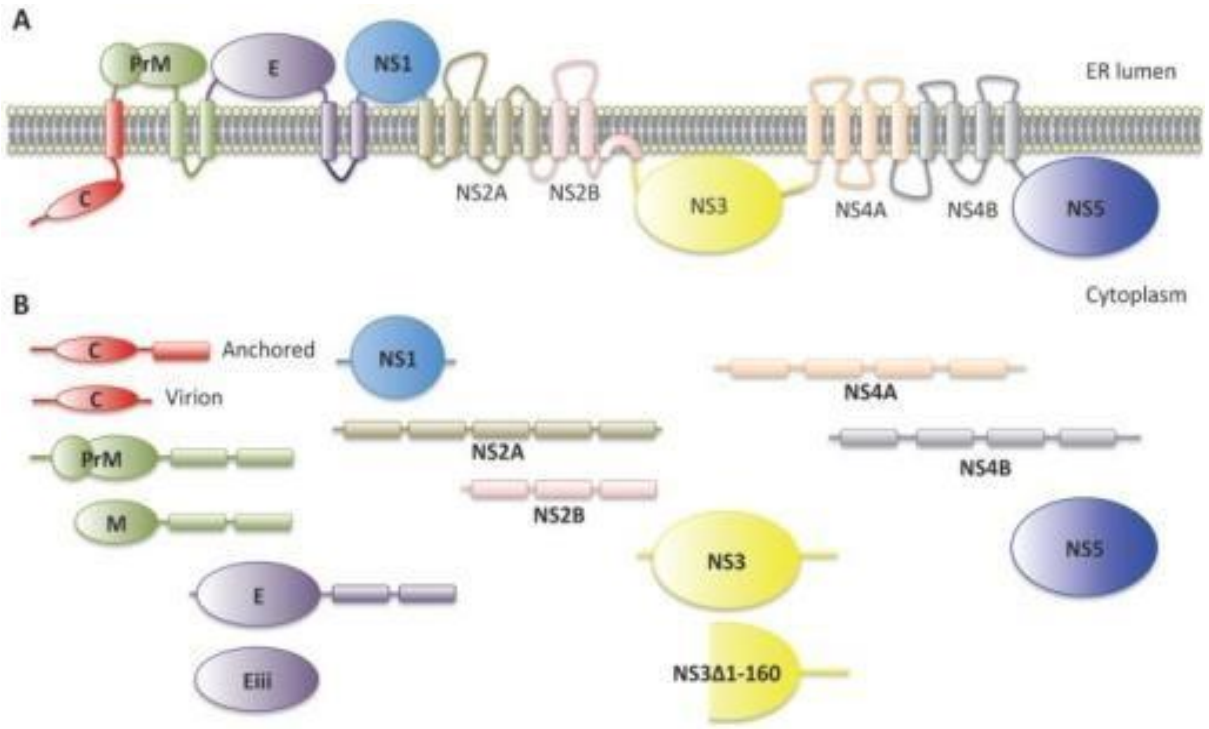


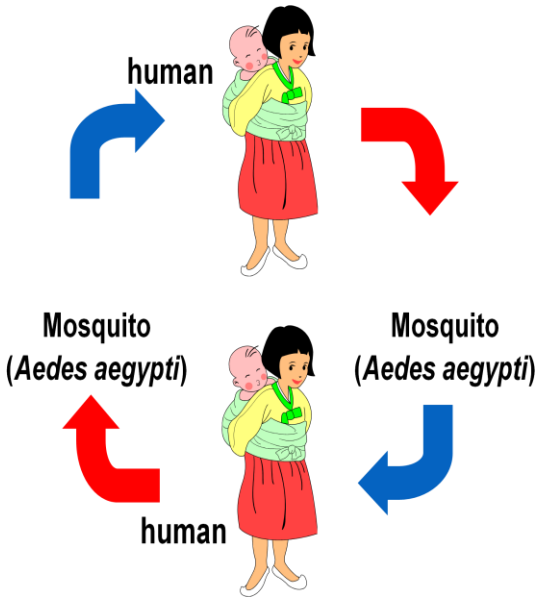
Figure 2.2.2: Dengue Structural and Non structural proteins (Mairiang, 2013).

Studies using cell culture have demonstrated that prM and E insert into the virion membrane to form the glycoprotein shell of the virus. During viral production and assembly, there is a complex series of rearrangements of prM and E. The virus is assembled in the endoplasmic reticulum, where 180 copies of both prM and E associate into trimeric spikes, each containing three prM and three E proteins (Modis, 2004). prM acts as a chaperone protecting the hydrophobic fusion loop of E from triggering premature fusion with host cell membranes. As the virion traffics through the Golgi apparatus, furin protease cleaves prM, and as the virion is secreted from the cell the cleaved pr polypeptide is released and the E protein rearranges into 90 dimers, giving a smooth mature virus particle (Mukhopadhyay, 2005). Following viral adhesion

to poorly characterized cellular receptors, the virus is endocytosed and acidification of the endocytic vesicle then triggers E to reassociate from dimers to trimers, which exposes the fusion loop, allowing the virion to fuse with the endocytic membrane, releasing the viral RNA into the host cell cytoplasm (Lin, 2008).

2.3 Dengue Transmission.

Dengue viruses are primarily maintained in a human-to-mosquito-to-human cycle (WHO, 2016).



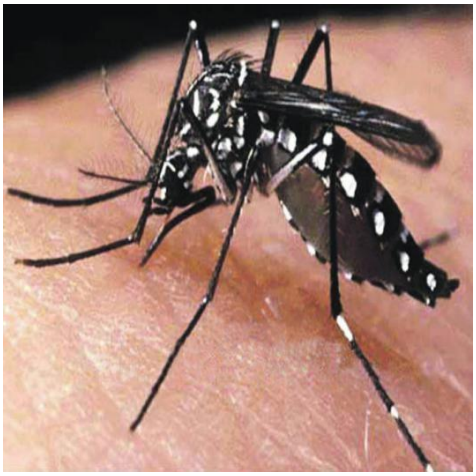


Figure 2.3.1: Dengue transmission cycle.

Figure 2.3.2: *Aedes aegypti*

Courtesy of James Gathany/PHIL/CDC.

Peak DENV infection occurs after period of increased rainfall due to increased multiplication of the mosquito vector, *Ae. aegypti* (El-Badry and Al-Ali, 2010) shown in Figure 2.6. *Aedes* mosquitoes are highly adapted to human habitation; they shelter indoors and bite during the daytime. *Ae. albopictus* can also sustain dengue virus transmission in humans. (WHO, 2016). Symptoms usually last for 5–10 days, after an incubation period of 4–10 days after the bite from an infected mosquito.

The spread of vectors following urbanization and the decline in vector-control efforts has partially contributed to the increased incidence of dengue virus infections. However, dengue is not confined to urban settings and is increasingly reported from rural areas. Additionally, factors such as population growth, globalization and travel, and climate change facilitate increased transmission of dengue viruses. (WHO, 2016).

2.4 Pathogenesis

Most infections by DENV occur after subcutaneous injection of the virus into the skin. When a mosquito (*Ae. aegypti* or *Ae. albopictus*), carrying DENV takes a blood meal, the virus enters the skin together with the mosquito's saliva. Released viral particles may infect nearby cells (thought to be predominantly monocytes or dendritic cells (DCs)) or activate resident immune cells such as mast cells. A local inflammatory response to DENV in the skin prompts the recruitment of leukocytes from the vasculature, including natural killer (NK) cells and T cells, which promote the killing of virus-infected cells at the site of injection. DENV is thought to then travel to draining lymph nodes via lymphatic vessels to establish systemic infection. These localized inflammatory responses occur many days before there are any signs of severe infection (John, 2013).

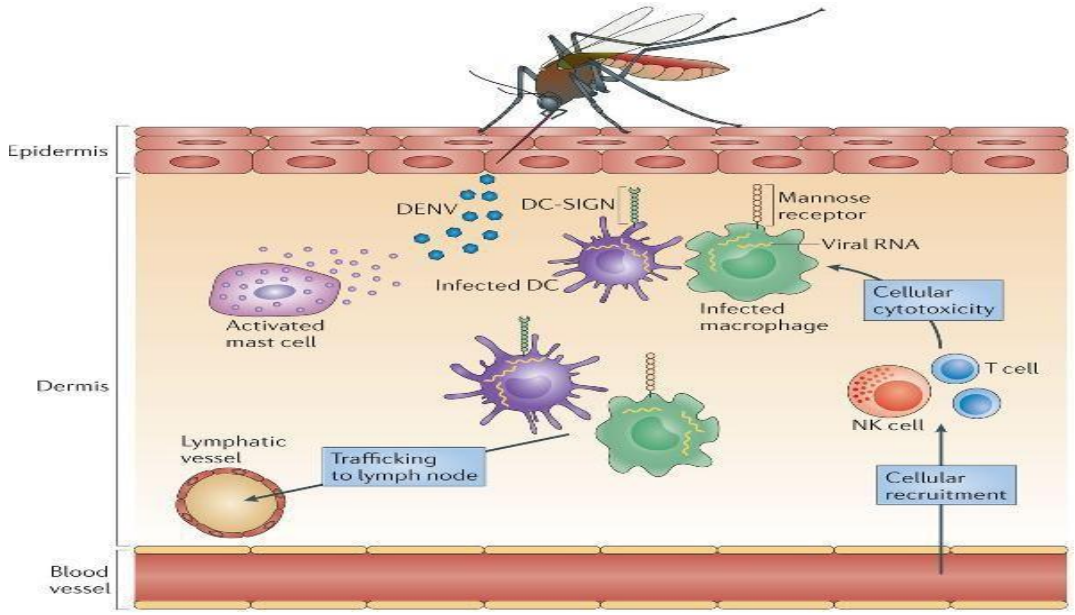


Figure 2.4.1 Host responses to cutaneous dengue virus injection (John *et al.*, 2011)

The immune cells respond by producing a number of signaling proteins, such as interferons and cytokines, which are responsible for many of the symptoms, such as fever, flu-like symptoms and plasma leakage. The amounts of virus circulating in patients are highly corelated with severe dengue, DHF and DSS (Noisakran et al., 2010). Understanding of manifested symptoms is paramount in classification of dengue whether as Dengue Fever or Dengue Haemorrhagic Fever of Dengue shock.

The virus production inside the body is greatly increased and many more organs (such as liver and bone marrow) can be affected with recent studies showing that hepatomegally is present between 50-100% of cases with elevated levels of enzymes aspartate minotranferase (AST) and alanine aminotransferase (ALT) which are believed to be sensitive indicators of liver damage (WHO, 2009). Plasma from the bloodstream usually leaks through the walls of the small blood vessels into body cavities due to the capillary cells fragility and permeability. Furthermore,

dysfunction of the bone marrow due to infection of the stromal leads to reduced numbers of platelets which are necessary for effective blood clotting. This increases the risk of hemorrhagic tendencies. As a result, less blood circulates in the blood vessels, and the blood pressure becomes so low that it cannot supply sufficient blood to vital organs. (Martina, Koraka, & Osterhaus, 2009).

2.5. Diagnosis of DF

DF is characterised by onset of fever above 38.5 °C, headache, retro-orbital pain, body aches, nausea and vomiting, joint pains, weakness and rash and may last 2 to 7 days. The fever may drop a few days later. DF is generally self limiting and is rarely fatal. The acute phase of illness lasts for 3 to 7 days but the convalescent phase may be prolonged for weeks and may be associated with weakness and prolonged depression (WHO, 1997). Blood specimens from patients with suspected DENV infections should be collected upon admission or attendance to the hospital.

2.6 Primary and Secondary Infections

The two basic methods for establishing a laboratory diagnosis of DENV infection will be detection of anti-dengue IgM and IgG antibodies. Immune response can be distinguished in two patterns: primary and secondary. Primary DENV infections typically have a stronger and more specific IgM response while the patients are still febrile. Detectable levels of IgM antibodies are noticed by day 5 to day 10 of illness using the IgM-capture ELISA. Once detectable, IgM levels rise quickly and appear to peak about 2 weeks after onset of symptoms; they then decline to undetectable levels over 2-3 months.. Anti-dengue IgG appears shortly afterwards. Primary infection is therefore characterized by the high anti dengue IgM and low anti dengue IgG (WHO,

1997). Figure 2.8 illustrates the host immune responses to dengue infection. Additionally, the duration of clinical symptoms is crucial in effective detection of dengue antibodies in patients.

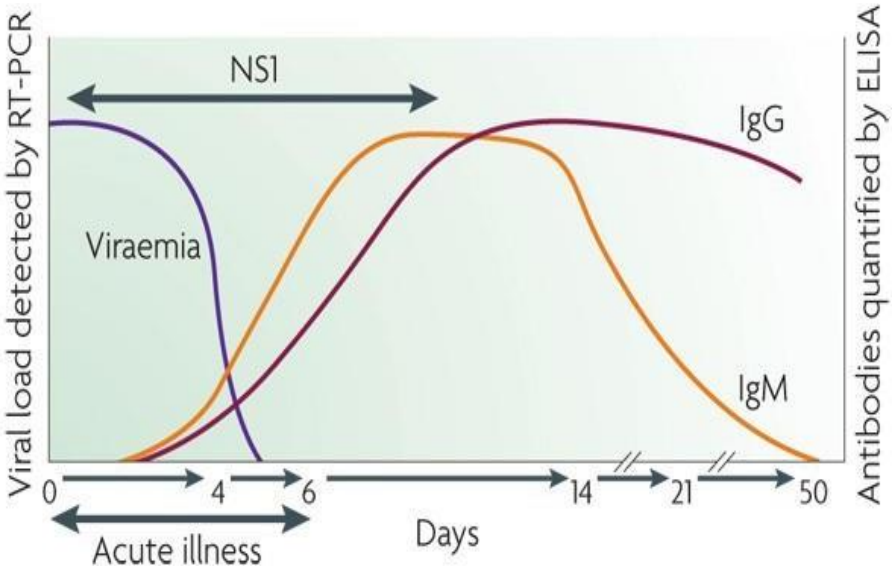


Figure 2.5.1. Immune response to dengue infection. (Guzman, 2010)

In contrast to primary infection, secondary infection with DENV results in the appearance of high levels of anti dengue IgG before or simultaneously with the IgM response. IgG levels rise quickly and peak about 2 weeks after the onset of symptoms while secondary infections show a weaker IgM response but a strong IgG response. The Indirect IgG ELISA is used to differentiate secondary infections from primary. It appears that secondary DENV infection with a different serotype of DENV places people at risk of DHF and DSS due to antibody-dependent enhancement phenomena (Rodenhuis-Zybert, et.al., 2010). ADE is observed when a person who has been previously infected with one serotype of DEV becomes infected many months or years later with a different serotype. Such cases have clinical course of the disease more severe and these people have

higher viremia since the previous non-neutralizing antiviral proteins facilitate virus entry into host cells, leading to increased infectivity in the cells (WHO, 2012).

2.7. Diagnosis of DHF and DSS

DHF shows manifestations of hemorrhage and plasma leakage which appear after fever remits. At the time of defervescence, hemorrhagic symptoms such as positive result of tourniquet test, bleeding gums, bleeding at venipuncture sites and purpuric lesions may occur from about 24hrs before or 24 hrs after temperature falls to normal or below. Complete Blood Count (CBC) tests usually show leucopenia (WBC count <4.0 DE x 10^9/L), thrombocytopenia (platelet count, <150 x 10^9/L) and hemoconcentration (hematocrit 20% increase from normal level). Thrombocytopenia is usually found between days 3 and 5 of illness. Thrombocytopenia may be associated with alterations in megakaryocytopoieses by the infection of human haematopoietic cells and impaired progenitor cell growth, resulting in platelet dysfunction (platelet activation and aggregation), increased destruction or consumption (peripheral sequestration and consumption). Haemorrhage may be a consequence of the thrombocytopenia and associated platelet dysfunction or disseminated intravascular coagulation. In summary, a transient and reversible imbalance of inflammatory mediators, cytokines and chemokines occurs during severe dengue, probably driven by a high early viral burden, and leading to dysfunction of vascular endothelial cells, derangement of the haemocoagulation system then to plasma leakage, shock and bleeding. (WHO, 2009).

Plasma leakage into extra vascular compartments usually increases due to vascular permeability leading to hemoconcentration and decreased blood pressure. Evidence of vascular plasma leakage

includes pleural or abdominal effusion on chest X-ray and abdominal ultrasound respectively and hemoconcentration by hematocrit. Without early diagnosis and proper management, some patients experience shock from the plasma leakage and blood loss which may be mild or severe characterized by low pressure and weak pulse. Some patients appear lethargic at first; they become restless and rapidly pass into a critical stage of shock. Skin may become cool and blotchy. The duration of shock is usually short and the patient may die within 8 to 24 hours (Gubler, 1998). Dengue symptoms that are used for classification are summarized in Figure 2.9 below.

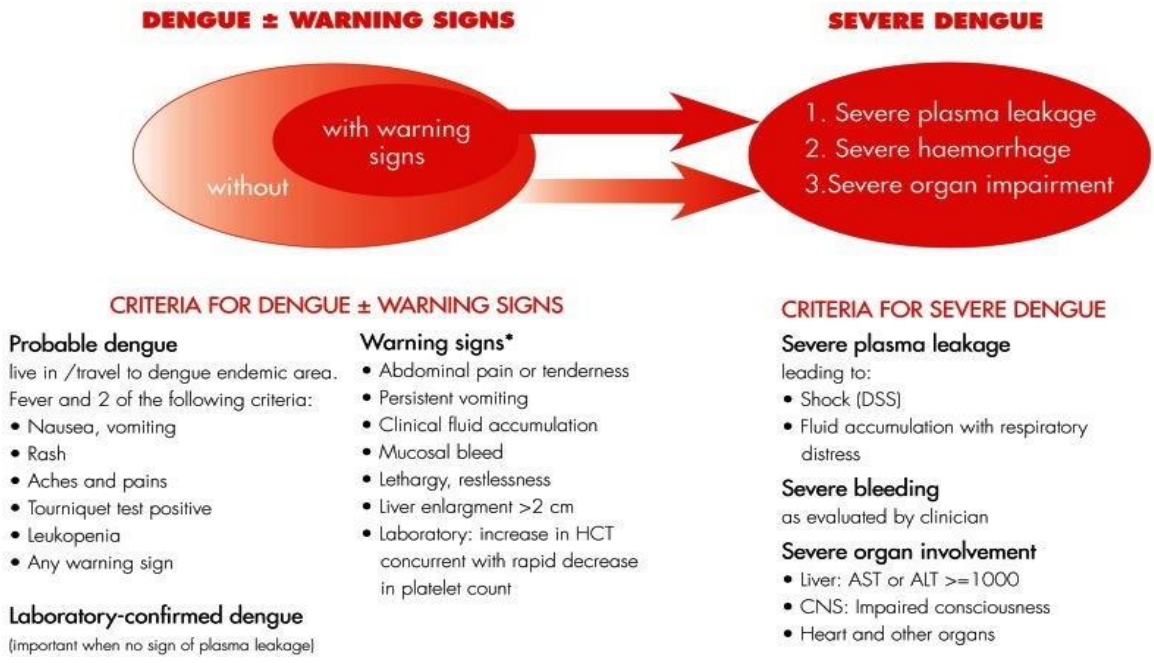


Figure 2.6.1 Dengue Case Definition (WHO, 2009)

2.8. Dengue Treatment

The current treatment of dengue relies on supportive measures with no direct therapeutics availabele (Yacoub, 2016). Management of dengue relies on supportive treatment in the form of

close monitoring for any of the "warning signs" and careful fluid balance for those identified to have capillary leak. Intravenous fluid is usually only required for patients with significant vascular leak and hemodynamic instability, or patients unable to tolerate oral fluids. The current WHO management guidelines recommend the initial use of crystalloid solutions, followed by colloid solutions for patients with profound or unresponsive shock. Patients may also be transfused if they lose excessive amounts of blood due to hemorrhage (WHO, 2009; Omuyundo,2014). Hospitalization is recommended for at least twenty-four hours following defervescence and patients should not be discharged until they meet the following criteria: return of appetite, clinical improvement, no respiratory distress, stable hematocrit, platelets greater than 50,000/mm^3, and good urine output (WHO, 2009; Omuyundo, 2014). DSS patients should remain hospitalized for at least two days following their recovery from shock (WHO, 2009; Omuyundo, 2014).In this study, DF admitted patients were observed daily to monitor for any signs and symptoms of DHF or DSS incidence.

2.9. Public Health Impact of Dengue

Inadequate public health planning and implementation of emergency control measures makes the impact of dengue amplified during outbreaks (Omuyundo, 2014). with the endmic nature of dengue at the Kenyan coast and circulating mosquito vectors, it can be perceived that secondary infections could occur in patients because of the four serotypes of DEV. Even though Dengue Haemorrhagic Fever and Dengue Shock Syndrome sometimes though seldom occur in primary infections, secondary infections have proven to increase the risk of DHF and DSS (Tantawichien, 2012). Predicting the development of DHF or DSS in a particular patient at the onset of dengue is difficult (WHO, 2012). Generally, most patients with dengue infections who exhibit warning signs and/or clinical features that are suggestive of the onset of DHF are usually

hospitalized (WHO, 2012). This situation is costly for socioeconomically disadvantaged facilities, such as CPGH, in which other health care resources must be delivered for management of dengue patients. This diversion of resources can potentially increase the suffering of patients with other illnesses. It is possible that the recognition of secondary dengue infection and the focused management of these patients might improve outcomes.

CHAPTER THREE

METHODOLOGY

3.1. Study Site

The study was done at the Coast Province General Hospital (CPGH) which is the biggest referral hospital in the entire Coast region of Kenya ($4^0$2'54''S, $39^0$40'26"E). The hospital serves urban population around Mombasa county as well as rural cases which come as referrals

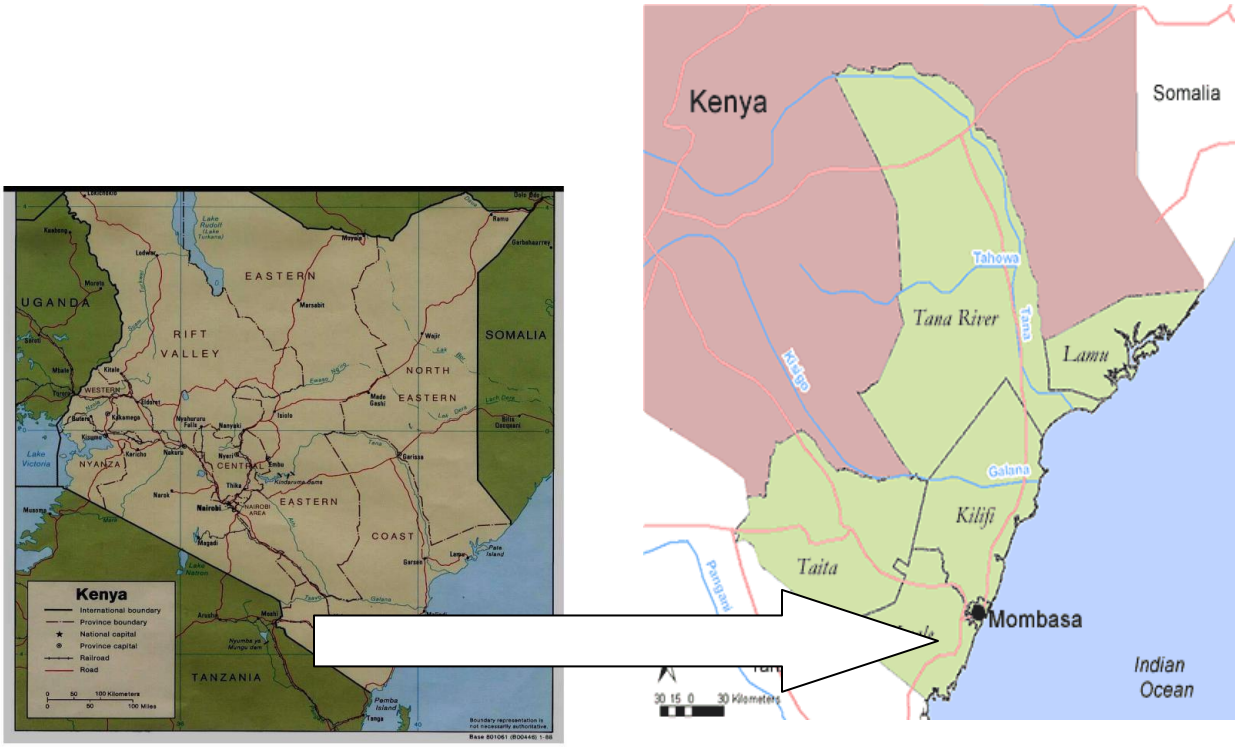


Figure 3.1.1 A map showing the coast region of Kenya courtesy of ©2013 ds-lands.com

The climatic conditions are generally hot and humid with two rainy seasons annually, March to April and October to November which serve as indicators for heightened vector breeding and increased cases of the endemic DF.

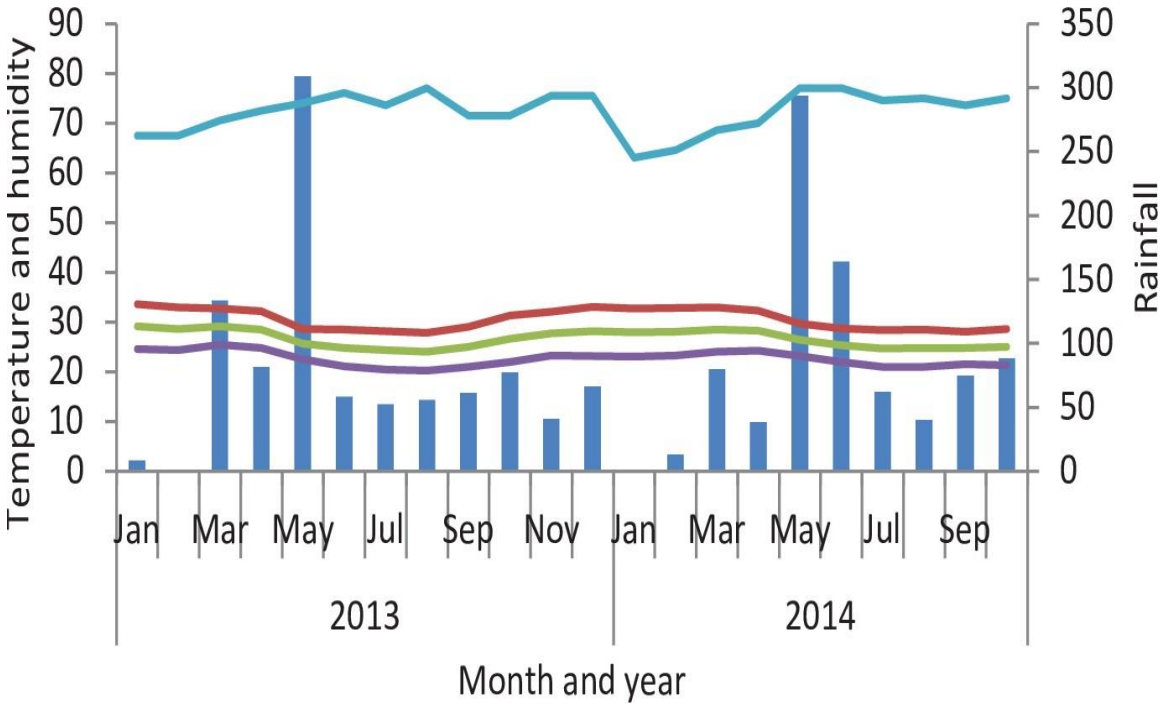


Figure 3.1.2 Rainfall and Temperature and relative humidity prevailing in Mombasa in 2013 and 2014 (Source: Kenya Meteorological Department, 2013 and 2014).

3.2 Sample Size Determination

A previous study by Omuyundo (2014) showed that there was a 13.9% dengue prevalence rate in Mombasa county alone between February and June of 2012. The sample size for this study was determined using Cochran formula with the prevalence of 13.9%. (Cochran 1977) since this it was the only published statistic at the time of sample size determination.

$$N = Z^2 P(1-P)D / d^2 .$$

Where;

N - Minimum number of sample required

Z - Standard normal distribution at 5% significance level (1.96)

P - Prevalence of DF for the coastal region (13.9%)

D - Design effect (1) d- Degree of precision (5%, 0.05)

$N = 1.96^2 (0.139)(1-0.139)\ 1 / 0.05^2 = 184$

+ 10% non corespondence

= 203 patients

3.3 Inclusion Criteria

1. Patients above 5 years with high fever (38.5 to 41.4^{o}C) and with two or more than the following symptoms; influenza-like symptoms, headache, joint pains, muscle pain, retro-orbital pain, abdominal pain, difficulty in breathing, rash, blood in stool, bleeding gums, nose bleeding and vomiting blood.

3.3.1 Exclusion Criteria

1. Pregnant women were excluded due to potential danger caused by the exposre to X-ray radiation for confirmation of pleural effusion where need be.

3.4 Enrollment and Data Collection Procedure

The study enrolled patients seeking medical care at CGPH outpatienbt and inpatient departments. The recruitment was done consecutively. The main purpose of conducting the research was explained to the study participants. Written consent was obtained from febrile patients, and parents or guardian of minors gave consent and the minors gave assent before recruitment into the study. Participation was voluntary and confidentiality was ensured by use of study numbers and the participant's name did not appear anywhere. Sociodemograhic components such as age, sex, occupation and marital status as well as clinical data were collected using a structured questionnaire.

The study clinician physically examined recruited patients making observations clinical manifestations and then 3mls of blood was drawn and aliquoted into yellow cap gel separator tubes for serological tests. IgM Capture positive samples were then tested for CBC after blood was collected in anticoagulant purple cap tubes. CBC and IgM Capture ELISA were carried out to confirm DENV infection. Indirect IgG ELISA was done to confirm secondary infection. AST and ALT levels were determined to assess liver damage. Admitted patients were observed daily. Chest Xray and abdominal ultrasound were done as per the advice of the clinician based on laboratory experiments and clinical manifestations suggesting chest or abdominal effusion.

3.4.1 Laboratory Experiments

All 203 participants provided samples for IgM capture ELISA, indirect IgG ELISA and CBC (including platelet count, haematocrit). Eight inpatients had liver enzymes (ALT and AST) levels tested. Non of the patients had chest X-ray or abdominal ultrasound done as it was not necessary.

3.4.1.1. IgM-capture ELISA

Recent DENV exposure was to be checked using IgM-capture ELISA following Bundo and Igarashi, 1985, protocol.

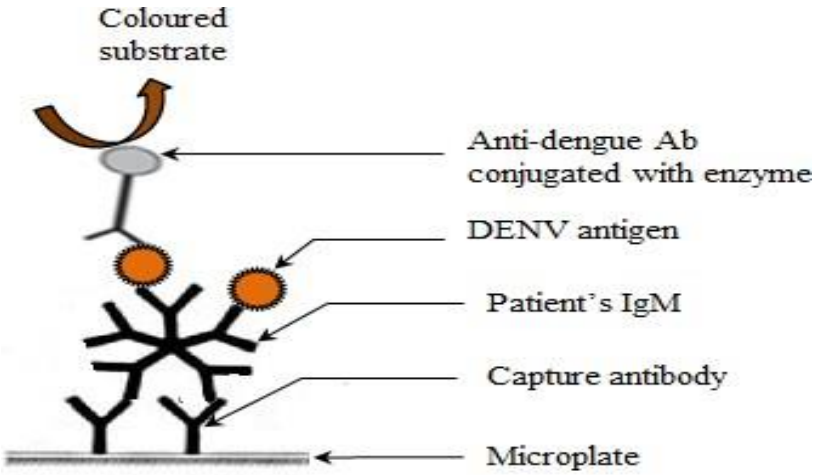


Figure 3.6.1.1: Illustration of IgM Capture Technique (Omuyundo, 2014)

Briefly, ninety six well microtitre plate was coated with 100 µl (5.5 µg/100 µl) of goat anti-human IgM (µ-chain specific) (Cappel ICN pharmaceuticals, Aurora, USA) diluted in ELISA coating buffer (0.05M carbonate-bicarbonate buffer, pH 9.6) in all wells except for the blank, and the coated plates incubated at 37 °C for 1hr. All wells except for the blank wells were blocked with 100 µl of blocking solution; blocked and incubated at room temperature ($24/25^0$C) for 1hr. Plate was washed X3 with PBS-Tween20. Duplicates of 100 µl positive control, negative control and patient serum sample diluted 1:100 using PBS-T (PBS containing 0.05% Tween20) were added to the plate, then incubated at 37 °C for 1 hr and the plate washed as described above. One hundred micro litres of DENV tetravalent antigen (100 ELISA units/100mL) were then added to each well except for the blank well as an assay antigen and the plate incubated at 37 °C for 1 hr. Plates were then washed as described above. One hundred microliters of 1:500

dilution of HRPO conjugated antiflavivirus monoclonal antibody (12D11/7E8) were added and the plate incubated at 37 °C for 1 hr. Washing was done as above. Colour was expected to develop after washing the plate and adding 100 µl of substrate: 5 mg *o*-phenylenediamine dihydrochloride (OPD) and 0.03 % hydrogen peroxide in 10 ml of 0.05M citrate-phosphate buffer at pH 5.0, to each well except for blank and the subsequent incubation in the dark at room temperature for 1hr. The reaction was stopped by adding 100 µl of 1N H_2SO_4 to each well. Optical density (OD) at 492 nm for each well was measured using Multiscan EX reader (Thermoscientific 355) using Skanit Software Version 3.1. A P/N (positive control (or sample) OD_{492}/negative control OD_{492}) ratio ≥2.0 was considered positive.

3.4.2. Indirect IgG ELISA

The IgG indirect ELISA was used for the detection of past DENV infections since IgG production is long term. This test was also to determine whether the patient is a case of primary or secondary DENV infection.

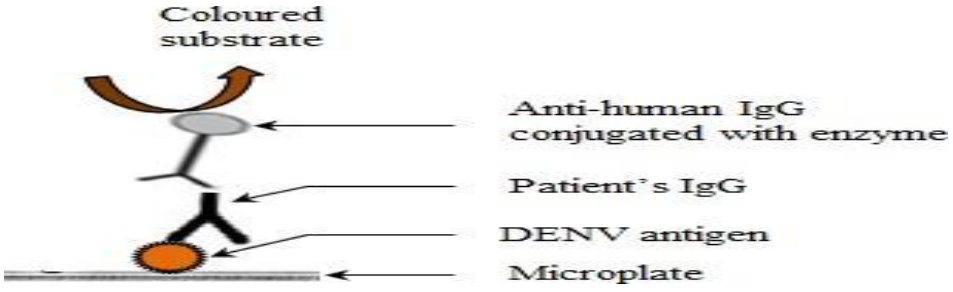


Figure 3.6.2.1: Illustration of Indirect IgG ELISA Technique (Omuyundo, 2014)

An in-house indirect IgG ELISA will be used for the detection of anti DENV IgG. A formalin inactivated sucrose-gradient purified antigen Japanese encephalitis virus vaccine strain (ML-17) was used as the assay antigen (Bundo *et.al* 1986). Ninety-six well flat-bottomed microtitre plates (Maxisorp, Roskilde, Denmark) were coated with a purified antigen (250 ng/100 µl/ well) with coating buffer (0.05M carbonate-bicarbonate buffer, pH 9.6) at 4 °C overnight. A hundred microlitres of Blockace (UK-B 80, Sapporo, Japan) was added to each well except the blank wells and the plate incubated at room temperature (r.t.) for 1 hr. The microtitre plates were then washed three times using PBS-T. Control and patient sample serum, 100µl of 1:1,000 dilutions in PBS-T with 10% Blockace, were added to each well in duplicate and incubated at 37 °C for 1 hr. This allowed the antibody to react with the antigen-coated wells.

The plates were washed as described above. A hundred microlitre of 1:4,000 diluted horseradish peroxidise (HRP)-conjugated goat anti-human IgG (American Qualex, California, USA) were added to each well and the plates incubated at 37 °C for 1 hr. The plates were then washed as described above. A hundred µl of substrate (5 mg *o*-phenylenediamine dihydrochloride (OPD) (Sigma Chemical, St. Louis, USA) and 0.03 % hydrogen peroxide in 10 ml of 0.05 M citrate-phosphate buffer at pH 5.0) were added to each well and subsequent incubation in the dark at room temperature for 20-30 min.

The reaction was stopped by adding 100 µl of 1N H_2SO_4 to each well. The optical density (OD) at 492 nm was measured using Multiscan EX reader (Thermo Scientific, Massachusetts, USA) using Skanit Software Version 3.1 (Thermo Scientific). Each sample was tested in duplicate, the mean OD for each sample measured and IgG titres calculated using a standard curve. The standard curve for each plate was prepared using the OD492 values of the

anti-dengue IgG high titre positive control serum starting with a 1000-fold dilution, followed by serial twofold dilutions up to $1:2^{13}$ in PBS-T+10% Blockace. Then, the IgG titers of patient sera were determined from the positive standard curve. A sample titer equal to, or greater than, 1:3000 (cut-off value of positive IgG) was considered IgG-positive for DENV (Inoue *et al*., 2010; Bundo *et al*., 1981).

3.4.3.1. Complete Blood Count

CBC including platelet count and haemotocrit levels were done using a full heamogram machine available at the hospital laboratory (Medonic Hematology Analyzer, Thermo Fischer Scientific Oy SN. 21478). Blood tests that show platelet count less that $150x10^9$/L showed patients manifestation of thrombocytopenia, leucopenia (WBC count <4.0 DE $x10^9$L and hemoconcentration (hematocrit 20% increase from normal level).

3.4.3.2. X-ray and Ultrasound Imaging

Evidence of plasma leakage where indicated was made through examination of pleural effusions observed through chest X-ray and/or abdominal effusion observed through abdominal ultrasound. With pleural effusion, fluid often builds up in the costophrenic angle (due to gravity). This can push the lung upwards resulting in blunting of the costophrnic angle. There needs to be atleast 75ml of pleural fluid in order to blunt the costophrenic angle on the lateral upright chest radiograph. Abdominal ultrasound may detect small volumes of fluid especially if its adjacent to the liver or diaphragm. Only patients who showed signs of pleural or abdominal effusion such as difficulty in breathing and abdominal pain were to have X-ray or ultrasound imaging.

3.4.5. Liver enzymes (ALT and AST) Levels

Liver enzymes, ALT and AST levels were tested to investigate the extent of liver damage tha was associated with DHF and DSS. Levels twice the normal levels (ALT , 40 units per litre and ALT 45 units per litre) were considered elevated and warning sign for DHF and DSS.

3.5 Statistical Analysis

Data wa entered into MS Exel 2007 and analyzed using IBM SPSS version 20. Percentage of DF cases among febrile patients was calculated to determine prevalence. Correltion analysis of anti-DENV IgM and anti-Flavivirus IgG among dengue cases was done to determine the percentage of primary and secondary infections. Incidence of DHF and DSS cases among dengue confirmed cases was determined using counts as they manifested any two of the following symptoms; difficulty in breathing, abdominal pain, nose bleeding, vomiting blood and blood in stool coupled with more than or equal 20 % increase in haematocrit levels. Twice the normal level of ALT (normal 40 units per litre) and AST (normal 45 units per litre) were considered elevated.

Categorical data such as age groups, area of residence, and types of infection (primary or secondary) were presented as frequencies. Pearson Chi square test was used to ascertain significance of association among these variables and the Phi and Cramer's V values used to measure the strength of tested association. A p value less than or equal to 0.05 was considered as statistically significant.

3.6 Measurement of Variables

IgM capture ELISA positive samples were considered current infections for calculation of prevalence of dengue. IgM positive samples that were also IgG positive were considered as secondary infections whereas those which were only IgM positive were primary infections. Samples which were only IgG positive were not considered as current dengue infections since

IgG are known to be antibodies are lifelong and indicate a previous infection. Warning signs that indicated that the patient could develop DHF and DSS were used as criteria for admission. These were IgM

positive samples mild thrombocytopenia (less than 100 x 10^9/L). Admitted patients were assessed for liver damage by testing ALT and AST enzyme levels.

3.7 Limitations of the Study

The study one major limitation; the republic of Kenya suffered a nationwide doctor's strike for the months of December 2016 to mid-March 2017. This grossly affected sample collection especially on the inpatient cases. With this in mind, the current prevalence of dengue could be predicted to be epidemiologically higher than reported. In addition, patients who were IgM negative but IgG positive were not considered as current infections for the duration of the study. The day of symptoms manifestation was put into consideration for this conclusion because immune responses and antibody titers depend on the incubation period of the virus from the time of the bite of a mosquito to onset of symptoms.

CHAPTER FOUR

RESULTS

4.1 Prevalence of Dengue Fever

The study period during the months of October 2016 to May 2017, a total of 203 samples from febrile patients were tested for dengue antibodies using an in-house IgM-capture ELISA and indirect IgG ELISA. Thirty six (17.7%) cases were confirmed as dengue infection while a hundred and seventy (82.3%) cases were found to be non-dengue. (Table 1) based on clinical manifestations and IgM-capture ELISA results.

Table 4.1.1. Prevalence of Dengue Fever

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Neg	167	82.3	82.3	82.3
	Pos	36	17.7	17.7	100.0
	Total	203	100.0	100.0	

Kisauni sub-county in Mombasa County recorded the highest number of patients in the study at 37.9 % and the least within the same county being Likoni at 6.4%. Among the dengue positive cases, Kisauni leads with 52. 8% followed by Changamwe sub-county at 13.9%. Sub-counties out of Mombasa including Garsen, Mwatate and Mariakani had the lowest number of patients at 0.5 % each. The association of sub-county of residence and infection was found to be insignificant ($p = 0.685$).

Table 4.1.2: Association Between Infection And Sub County Of Residence

	Value	df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)		
				Sig.	99% Confidence Interval	
					Lower Bound	Upper Bound
Pearson Chi-Square	13.740[a]	17	.685	.676[b]	.664	.688
Likelihood Ratio	15.548	17	.556	.625[b]	.612	.637
Fisher's Exact Test	12.754			.759[b]	.748	.770
N of Valid Cases	203					

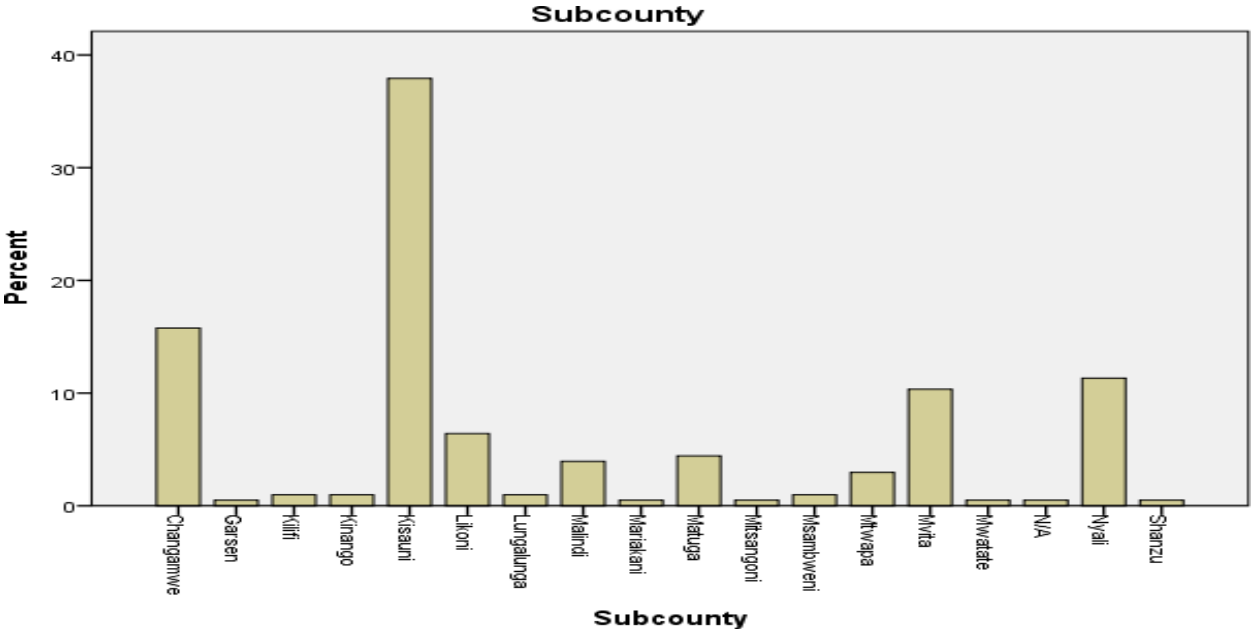


Figure 4.1.3: Graph Showing the Percentage of Patients Per Sub-county

4.2. Primary and secondary infections.

It was observed that 21 out of the 203 patients (10.3%) were primary infections whereas 15 (7.4%) were secondary infections. The patients were categorized into age groups and Pearson Chi square test analysis was done against the types of infection (whether primary or secondary) to test for association. The strength of association was measured by Phi and Cramer's V values.

Table 2 below shows that primary infections were highest (4.5%) in the 30-44 years age group while secondary infections were highest (4%) in the 15 -29 years age group.

Table 4.2.1.Age Group and Type_of_Infection Cross tabulation

		Type_of_Infection			Total
			P	S	
0-14	Count	43	3	6	52
	% within 1	82.7%	5.8%	11.5%	100.0%
	% within Type_of_Infection	25.9%	15.0%	40.0%	25.9%
	% of Total	21.4%	1.5%	3.0%	25.9%
15-29	Count	31	5	8	44
	% within 1	70.5%	11.4%	18.2%	100.0%
	% within Type_of_Infection	18.7%	25.0%	53.3%	21.9%
	% of Total	15.4%	2.5%	4.0%	21.9%
30-44	Count	76	9	1	86
	% within 1	88.4%	10.5%	1.2%	100.0%
	% within Type_of_Infection	45.8%	45.0%	6.7%	42.8%
	% of Total	37.8%	4.5%	0.5%	42.8%
45 -59	Count	14	3	0	17
	% within 1	82.4%	17.6%	0.0%	100.0%
	% within Type_of_Infection	8.4%	15.0%	0.0%	8.5%
	% of Total	7.0%	1.5%	0.0%	8.5%
60-74	Count	2	0	0	2
	% within 1	100.0%	0.0%	0.0%	100.0%
	% within Type_of_Infection	1.2%	0.0%	0.0%	1.0%
	% of Total	1.0%	0.0%	0.0%	1.0%
Total	Count	166	20	15	201
	% within 1	82.6%	10.0%	7.5%	100.0%
	% within Type_of_Infection	100.0%	100.0%	100.0%	100.0%
	% of Total	82.6%	10.0%	7.5%	100.0%

Ho Type of infection and age group are independent

H_1 Type of infection and age group are not independent

Table 4.2.2. Association between Age-group and Type of Infection

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.366[a]	8	.027
Likelihood Ratio	19.398	8	.013
N of Valid Cases	201		

The type of infection and age group are not independent, p= 0.027 and the strength of association is high. (Phi and Cramer's V = 0.027)

4.3 Investigation of Incidence of DHF and DSS

Anti-dengue IgM positive patients were investigated for warning signs for DHF and DSS which include thrombocytopenia, hemoconcentration and liver enzymes AST and ALT. Patients that showed warning signs were admitted and observed daily.

4.3.1 Thrombocytopenia

Decreased in platelet level was considered as one of the warning signs for admission. Patients with platelet count less that 150 x 10^9 /L were noted as having thrombocytopenia. 8 (3.9%) of the sample size were admitted. 3 inpatients (1.5% of sample size) had severe thrombocytopenia (<50 x 10^9/L) but they did not progress to bleeding manifestations.

4.3.2. Liver Enzymes (ALT and AST) Levels

The normal range for AST was reported to be between 10 to 45 units per litre and ALT between 10 to 40 units per liter. Generally, mild elevations are considered to be 2 to 3 times higher than the normal range. The 8 Inpatients were tested for these enzymes. The enzymes were slighlty elavated with the highest ALT levels recorded as 62 units per litre and AST as 63 units per litre.

4.3.3. Hemoconcentration

Normal hematocrit levels ranged from 35 -55%. A 20% increase in hematocrit levels was considered a sign for DHF or DSS. The hematocrit values ranged from 33.6 % as the lowest to 50.1% as the highest value. These values did not surpass the normal levels and therefore did not indicate hematoconcentration which is a critical sign in severe dengue. Out of the thirty six IgM-capture positive patients, eight (22.8%) were inpatients. Since there is no direct medication for dengue, patients were given supportive treatment including paracetamol and IV fluids (normal saline ans Ringers solution). The average day of discharge from hospital was four.

4.3.4. DHF and DSS Clinical Manifestations

	Cases					
	Valid		**Missing**		**Total**	
	N	**Percent**	**N**	**Percent**	**N**	**Percent**
IgM_result * Difficulty_in_breathing	203	100.0%	0	0.0%	203	100.0%
IgM_result * Melena	203	100.0%	0	0.0%	203	100.0%
IgM_result * Bleeding gums	203	100.0%	0	0.0%	203	100.0%
IgM_result * Nose Bleeding	203	100.0%	0	0.0%	203	100.0%
IgM_result * Vomiting_blood	203	100.0%	0	0.0%	203	100.0%

None of the patients fit the clinical manifestations criteria for DHF and DSS.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of Dengue Infection Cases among Febrile Patients

During the study period, October 2016 to May 2017, the prevalence of dengue was recorded as 17.7%. This is an increase from 13.9% in 2012 (Omuyundo, 2014) and 15% in 2014, 2015 (Munyuga et.al. 2016). The increase could also be attributed to the dengue fever outreak that was reported between the months of March and May 2017 in Mombasa.

Generally, Mombasa city experiences a tropical wet and dry climate (Kotek, 2006). The amount of rainfall is dependent on the season with April and May (long rain season) receiving the highest amount, October to December (short rain season) moderate amount while January to February is the driest and receives minimal amount of rainfall. (Lutomiah, *et.al.*, 2016). The study was conducted during the two peak seasons of dengue in Mombasa. Circulationg of the vector mosquito in Mombas may have epidemiological significance with regard to the maintenance of DENV in nature in conditions adversarial to the virus. The coastal region in Kenya is usually characterized by high temperatures, throughout the year, that favor the proliferation of DENV and subsequent transmission by *Ae*. *Aegypti*.(Chepkorir, 2014)

Slightly above half the number of dengue positive cases (52.8%) reside in Kisauni subcounty which is the largest subcounty in Mombasa covering 109.7km2. Changamwe subcounty in Mombasa follows at 13.9%. However, there was no siginifant statistical association between place of residence and infection.(p=0.685). Kisauni subcounty is densely populated and is mostly affected by floods during the rainy season due to ineffective drainage systems creating suitable habitats for the vector mosquitoes. The high population enhances spread of dengue in households.

5.2. Primary and Secondary Infections

The patients were diagnosed for primary DENV infection, secondary DENV infection and non-dengue infection depending on IgG antibody titer against DENV**.** Out of the thirty six dengue confirmed cases, 21 (10.3%) were primary infections whereas 15 (7.4%) were secondary infections. Primary infections were highest (4.5%) in the 30-44 age groups whereas secondary infections were highest (4%) in age group 15-29. The association between type of DENV infection by age groups was statistically significant ($p = 0.027$) with a strong association (Phi and Cramer's V value = 0.027). The present findings may be explained by the fact that the age groups 30 t0 44 years and 15 to 29 years are predominantly outdoors during the day whether in school or in occupational places giving chance for the day biting vectors to transmit the virus. Dengue infection is generally considered to be a paediatric disease but is currently a growing problem in adults throughout the tropics. The age distribution of dengue has been rising and more cases have been observed in adolescents and adults. (Tantawichien 2012). Furthermore, dengue infection can be more severe in adults in whom early recognition of dengue infection, bleeding tendencies and signs of circulatory collapse would reduce mortality.

5.3 DHF and DSS

It can be concluded that there was no case of DHF or DSS among the patients attending the Coast Provinve General Hospital during the study period. Despite the occurrence of the dengue outbreak, none of the patients showed manifestations of severe dengue. Out of the thirty six dengue confirmed cases, eight were inpatients who had shown warning signs for DHF and DSS and whose average day of discharge form the hospital was four. The inpatients were managed using paracetamol drugs, IV fluids (normal saline and Ringers solution) and multivitamins. This means that the medical staff were in line with the WHO recommended management of dengue criteria.

(WHO, 2009). All inpatients recovered and were allowed to go home. This shows that, with adequate supportive care, progression of DF to DHF and DSS can be averted. Absence of DHF and DSS among these patients could be better understood by exploring the epidemiology of dengue infections with regard to the susceptibility of African populations to dengue and the interference between dengue and the other major communicable diseases of the continent e.g HIV/AIDS and Malaria.(WHO, 2009). Recent studies in the same region by Omuyundo 2014 and Munyuga 2016 also could not establish DHF and DSS among the samples assessed as they did not go furthure to investigate the posibility.

CHAPTER SIX

SUMMARY, CONCLUSION, RECOMMENDATION

6.1 Summary

Dengue is one of the world's most rapidly emerging diseases, and as incidence continues to rise in endemic areas, and transmission in new regions of the world becomes established, there are major public health challenges ahead. Major risk factors for DHF and DSS are mosquito vector population and secondary infection of DENV 1- 4.This study was conducted from October 2016 to May 2017 presumed to cover dengue peak seasons with a total of 203 samples. DENV specific IgM and flavivirus IgG antibodies were determined using an in-house ELISA. 36 (17.7%) out of 203 febrile cases were positive for anti-DENV IgM. Of the 36 positive cases, 21 (10.3%) were primary infections and 15 (7.4%) were secondary infections. Of the dengue positive cases, none could fit the criteria for DHF/DSS. The relationship between age groups and type of infection; whether primary or secondary, was found to be significant (p=0.027) with primary infections highest (4.5%) in the 30-44 age groups whereas secondary infections were highest (4%) in age group 15-29. Dengue infection did not have a significant association with subcounty of residence (p = 0.685). The study reported a dengue prevalence rate of 17.7%, an increase from previous years showing the endemic nature of dengue.

6.2 Conclusion

With the data from the study, it can be concluded that;

i) Dengue prevalence continues to rise annually in Mombasa.

ii) The age distribution of dengue primary and secondary infections has been rising and more cases have been observed in adolescents and adults.

iii) There was no incidence of DHF and DSS during the study period.

6.3. Recommendation

1. Continued surveillance of dengue in public and private health facilities is needed for a clearer picture on prevalence of the disease especially in peak seasons.

2. The population, especially adolescents and adults should be enlightened about personal protection as envrinmental control measures to lower transmission rates.

3. DF patients who show warning signs for DHF and DSS should be admitted and given supportive treatment to avert the progression to DHF and DSS

6.4 Recommendations for Future Studies

Research on susceptibility of the African population to severe dengue in relation to other disease such as malaira and HIV/AIDS is recommended to cover knowledge gaps in dengue research. Furthermore, being a tourist destination, screening of travellers in the country's entry points should be included in surveillnce especially before peak seasons of the disease.

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APPENDICES

APPENDIX I: INFORMED CONSENT FORM

ASSESSMENT OF THE EXISTENCE OF SEVERE DENGUE VIRUS INFECTION AMONG PATIENTS ATTENDING THE COAST PROVINCE GENERAL HOSPITAL.

Investigators

1. Nancy Nzalambi Kuttoh, Masters of Public Health, Maseno University.
2. Dr. Guyah Bernard, School of Public Health and Community Development, Maseno University.
3. Dr. Shingo Inoue, Kenya Medical Research Institute/Nagasaki University.

CONSENT FORMS FOR ADULTS

Project title: Assessment of the Existence of Severe Dengue Virus Infection Among Patients Attending the Coast Province General Hospital.

Name of health facility ……

Study number: …….. Date of Admission …….

Introduction: Dengue is an important cause of febrile illness in this area. Dengue is categorized into acute dengue fever and severe conditions of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). We are conducting a study to analyze dengue virus infections including severe cases of dengue (DHF and DSS) among patients attending the Coast Province General Hospital. Information we gather is useful to policy makers who may consider better patient management programs in this community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including Ministry of Health, Maseno University, KEMRI, and others. The Maseno University Ethical Review Committee has approved this study.

Research Procedures: If you agree to be a participant in this study, we will ask you a few questions regarding the status of illness. Then 3ml of blood will be drawn for complete blood count and checking dengue antibody status. Sterile and disposable instruments that are clean and safe will be used. Chest and or abdominal X-ray will be performed on you upon advice by the clinician. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but a study number assigned to you will be used to label the sample. It may be necessary to take the blood sample taken from you to a laboratory in the country for further tests.

Risk/benefits: During this procedure there will be no long-lasting effect. However, you may feel a brief moment of pain, discomfort or fear. You will not be given any monetary benefits; neither will you incur any costs. The study will benefit your community since by helping us analyze the real picture of clinical severity of dengue virus infection in your community and we will be able to recommend and design appropriate interventions in management of this disease.

Participant's Rights: Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Confidentiality: You will not be identified by name in any report or publication of this study or its results. Your sample will be identified using a study number assigned above.

Contact Information: If you have questions now or in the future regarding your rights or this study, you may ask any of the field officers involved in this study or contact Nancy Kuttoh of Maseno University via 0774 170 765. You can also contact Maseno University Ethical Review Committee at muerc-secretariate@maseno.ac.ke or +254 57 351 622 EXT 3050.

May I now ask if you would like to participate in the study?

The above details about the study and the basis of participation have been explained to me and **I agree** to take part in the study. I understand that I am free to choose to be part of the study. I also understand that if I do not want to go on with the study, I can withdraw at any time. **I give my consent** for diagnosis of dengue fever, dengue hemorrhagic fever and dengue shock syndrome.

Please sign here or put your right hand thumb mark if you agree:

Name: ----------

Signature/ Thumb mark---------- Date ----------

If the patient is illiterate, an adult must witness the consent process

Name of Witness: ---------- Age----------

Signature of Witness: ---------- Date: ----------

Name of Investigator: ----------

Signature of Investigator: ---------- Date: ----------

CONSENT FORMS FOR MINORS

Project title: Assessment of The Existence of Severe Dengue Virus Infection Among Patients Attending the Coast Province General Hospital.

Name of health facility ……

Study number: ……Date of Hospital Visit: …….

Introduction: Dengue is an important cause of febrile illness in this area. Dengue is categorized into acute dengue fever and severe conditions of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). We are conducting a study to analyze dengue virus infections including severe cases of dengue (DHF and DSS) among patients attending the Coast Province General Hospital. Information we gather is useful to policy makers who may consider better patient management programs in this community or other communities in the future. We will summarize our findings from this study and disseminate it to various stakeholders including Ministry of Health, Maseno University, KEMRI, and others. The Maseno University Ethical Review Committee has approved this study.

Research Procedures: If you agree that your child will be a participant in this study, we will ask you a few questions regarding the status of illness. Then 3ml of blood will be drawn for complete blood count and checking dengue antibody status. Sterile and disposable instruments that are clean and safe will be used. Chest and or abdominal X-ray will be performed on your child upon advice by the clinician. In order to ensure complete confidentiality of the test results, no names will be attached to the blood samples, but a study number assigned to your child will be used to label the sample. It may be necessary to take the blood sample taken from your child to another laboratory in the country for further tests

Risk/benefits: During this procedure there will be no long-lasting effect. However, your child may feel a brief moment of pain, discomfort or fear. Your child will not be given any monetary benefits; neither will you incur any costs. The study will benefit your community since by helping us analyze the real picture of clinical severity of dengue virus infection in your community and we will be able to recommend and design appropriate interventions in management of this disease.

Participant's Rights: Your approval for your child's participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you or your child.

Confidentiality: The child will not be identified by name in any report or publication of this study or its results. Your child's sample will be identified using a study number assigned above.

Contact Information: If you have questions now or in the future regarding your rights or this study, you may ask any of the field officers involved in this study or contact Nancy Kuttoh of Maseno University via 0774 170 765.

May I now ask if you would like your child to participate in the study?

The above details about the study and the basis of participation have been explained to me and **I agree that my child** will take part in the study. I understand that I am free to choose to be part of the study. I also understand that if I do not want my child to go on with the study, I can withdraw at any time. **I give my consent** for diagnosis of dengue fever, dengue hemorrhagic fever and dengue shock syndrome.

Please sign here or put your right hand thumb mark if you agree:

Name: ----------

Signature/ Thumb mark---------- Date ----------

If the patient is illiterate, an adult must witness the consent process

Name of Witness: ---------- Age----------

Signature of Witness: ---------- Date: ----------

Name of Investigator: ----------

Signature of Investigator: ---------- Date: ----------

APPENDIX II: QUESTIONNAIRE

This questionnaire is to be administered to patients attending the hospital.

Clinic Code: …….

Clinic/ health facility name ……

Date of hospital visit ……

Study Number ……..…….

Village of residence ……...

Ward..... ……..

Subcounty ……..

County ……..

Demographic characteristics:

Sex: Male( ) Female ( )

If female; is there possibility of pregnancy?

Yes ( ) No ( )

Age (years)( ) Date of birth: Day( ) Month( ) Year( )

Social characteristics:

Marital status: Single ( ) Married ( )

Occupation: …….

If child is school going, which school is the child attending?

…….

Have you ever been vaccinated against Yellow Fever?

Yes ( ) No ( )

If Yes, when: Year ( ) Date ( ) Age at the time of vaccination ( )

Clinical signs and symptoms:

Fever:

Yes ( ) No ( )

Duration of fever ……..

Influenza like symptoms:

Yes ( ) No ( )

Duration of symptoms……..

Headache:

Yes ( ) No ( )

Duration of symptoms (in days)……..

Joint pains:

Yes ( ) No ( )

Duration of joint pains (in days)……..

Muscle pain:

Yes ( ) No ( )

Duration of muscle pains (in days)……..

Pain behind the eyes:

Yes ( ) No ( )

Duration of symptoms (in days)……..

Abdominal pain:

Yes ( ) No ( )

Difficulty in breathing:

Yes ( ) No ( )

Duration of symptoms (in days)……..

Rash:

Yes ( ) No ( )

Duration of symptoms (in days)……..

Bleeding tendencies:

Yes ( ) No ( )

Blood in stool:

Yes ( ) No ( )

Duration of symptoms ……..

Bleeding gums:

Yes ( ) No ( )

Duration of symptoms ……..

Nose bleeding:

Yes ( ) No ( )

Duration of symptoms ……..

Vomiting blood:

Yes ( ) No ( )

Duration of symptoms ……..

Any drugs taken within the period of illness:

Yes ( ) No ( )

If yes, which ones ……..

Previous experience of above symptoms:

Yes ( ) No ( )

How long ago?……..

Tourniquet test:

Positive ( ) Negative ( )

List any other symptoms

1. …….
2. …….
3. …….

APPENDIX III: PATIENT OBSERVATION CHART

Project Title: Assessment of the Existence of Severe Dengue Virus Infection Among Patients Attending the Coast Province General Hospital

Conducted by Ms. Nancy Kuttoh (MPH Student, Maseno University)

Patient Observation Chart

Hospital ID Number:

Study ID Number:

Ward Number:

Bed Number:

Degree of symptom: None (-), Yes Mild (+), Intermediate (++), Severe (+++)

Period of illness	**Date**							
	Day							
	Days from admission	1	2	3	4	5	6	7
General Symptoms	**Fever**							
	Temperature							
	Headache							
	Dizziness							
	Fatigue							
	Restlessness							
	Loss of appetite							
	Vomiting							
	Nausea							
	Muscle Pain							

	Joint Pain							
	Backache							
	Diarrhea							
Hemorrhagic manifestations	**Rash**							
	Tourniquet test							
	Epistaxis (nose bleeding)							
	Gum bleeding							
	Vomiting blood							
	Hematemesis							
	Melena							
Plasma Leakage	**Abdominal pain**							
	Difficult breathing							
	Pale eyes							
	Clammy skin							
	Pale mucus membrane							
	Weak pulse							
Other symptom	**Hepatomegaly**							
	Jaundice							
TREATMENT	**Anti-febrile drug**							
	transfusion							
	Other medicine							
NOTE								

APPENDIX IV: MASENO UNIVERSITY ETHICAL REVIEW FORM

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

FROM: SECRETARY - MUERC **DATE**: 27th April, 2016

TO: Nancy Nzalambi Kutto h **REF**: MSU/DRPI/MUERC/00282/16
EL/ESM/00596/2013
Department of Public Health
School of Public Health and Community Development
Maseno University

RE: Proposal Reference Number MSU/DRPI/0282/16 **"Assessment of the Existence of Severe Dengue Virus Infection among Patients attending the Coast Province General Hospital".**

MUERC is pleased to inform you that your proposal application was reviewed and discussed in the Committee meeting held on 21st April, 2016.

In its review the Committee noted the following **Minor Corrections** to be made before Ethics Clearance is granted:

i. Explain how you intend to safely store the biological samples.
ii. Ethical issues are not addressed. Explain the ethical issues that shall be addressed especially in data collection, storage and use.
iii. Also explain how you intend to protect the rights of the study participants.
iv. Ethical approval will be sought from MUERC and not SGS.
v. Include MUERC contacts in the consent form.
vi. References, work plan and budget to come after the methodology, the other appendices to continue.

The Committee granted the Investigators thirty (30) working days to make corrections and submit a final draft proposal to MUERC Secretariat for consideration and approval.

Please submit **one copy of corrected draft proposal** and a **signed cover letter,** detailing the sections (page Numbers and paragraphs) where corrections are made. Include your **proposal Ref Number** on the cover letter

Thank you.

Yours faithfully,

Dr. Bonuke Anyona
Secretary - MUERC
Cell phone: +254 721 543 976; +254 733 230 878
Email: sbonuke@gmail.com

APPENDIX V: HOSPITAL APPROVAL

MINISTRY OF HEALTH

Telegrams: "MEDICAL", Mombasa
Phone: Mombasa 2314202/5, 2222148, 2225845
Fax: 2220161 E-mail: chiefadmin@cpgh.co.ke
Address all correspondence to the Chief Admin.
When replying, please quote Ref. No. & date.

COAST PROVINCE GENERAL HOSPITAL
P.O. BOX 90231
MOMBASA

Ref. No. MED.4/II/VOL.I/49 **Date: 30th June 2016**

Nancy Nzalambi Kuttoh
P O Box 81-80114
MAZERAS

RE: RESEARCH PROPOSAL: ASSESSMENT OF THE EXISTENCE OF SEVERE DENGUE VIRUS INFECTION AMONG PATIENTS ATTENDING THE COAST PROVINCIAL GENERAL HOSPITAL

This is to inform you that the CPGH Ethics & Research Committee has reviewed and approved your above proposal starting from 1st July 2016 to 30th June 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by CPGH-ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the CPGH-ERC within 72 hours of notification
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to CPGH-ERC within 72 hours
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period (attach a comprehensive progress report to support the renewal)
f) Clearance for export of biological specimens must be obtained from CPGH-ERC for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

DR. M. OCHOLA
SECRETARY, CPGH-ERC

C.C - Chairman – ERC
- Chief Administrator – CPGH
- Director of Nursing
- Maseno University
- Director of Support Services.