PREVALENCE OF DENGUE VIRUS IgM IN BLOOD SAMPLES OF PATIENTS FROM DIFFERENT STATES IN SOUTHWESTERN NIGERIA USING ELISA KIT

By

OGUNLEYE BOSEDE BOLAJI

MATRIC NO/11/0340

A DISSERTATION

IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCE [B.Sc.] degree SUBMITTED TO THE DEPARTMENT MICROBIOLOGY, FACULTY OF SCIENCE.

FEDERAL UNIVERSITY OYE EKITI,

EKITI STATE, NIGERIA

SUPERVISOR, DR OKOROR LAWRENCE

OCTOBER 2015

CERTIFICATION PAGE

We satisfy that this project work was presented by OGUNLEYE BOSEDE BOLAJI of the Department of Microbiology, Faculty of science, Federal University Oye- Ekiti. Under the supervision of DR OKOROR .L

25/10/15

.....

DR OKOROR .L

[PROJECT SUPERVISOR]

.....

DATE

.....

PROF.B.O OGENEH

[HEAD OF DEPARTMENT]

17/11/15

.....

DATE

DEDICATION

I dedicate this project work to the glory of Almighty God .The source of my wisdom and good health throughout my staying in the university and also to parent, friends and family for their support and their prayers.

ACKNOWLEDEGEMENT

My greatest gratitude is directed to Almighty God for sparing my life and seeing me through my project in a hitch free and conducive atmosphere.

My heartfelt gratitude goes to my parent whom I received their full support, prayers and financial support throughout my project. I am also grateful to my siblings for their support and prayers.

Profound thanks also go to my supervisor Dr. OKOROR. L .who in the midst of no time and space prepared for me a worthwhile Laboratory working experience. Also my appreciation goes to Head of department, PROF B.O OGENEH, my lecturers and the entire technologist.

This acknowledgement remains incomplete without expressing my profound gratitude to my friends, course mates, and to pastor Ajewole and his wife for the wonderful inspiration, prayer and encouraging words during my project. Thank u all

I LOVE U ALL.

TABLE OF CONTENT

CHAPTER FOUR [RESULT]

CHAPTER FIVE [CONCLUSION AND RECOMMEDATION]

LIST OF TABLES

LIST OF PLATES

ABSTRACT

Dengue virus has been classified in the past years as a member of the family Flaviridea, species name dengue virus .it is one of the most important vector borne disease of modern man with about 50million people infected per year. The experiment was conducted with the aim to detect the prevalence of dengue virus IgM among patients attending clinic for typhoid and malaria in Ekiti, Ondo, and Osun states .The objective was to determine the prevalence of dengue virus IgM antibodies in these population .This study was carried out using 27 blood samples collected from patients [adult and children] reporting for typhoid and malaria from different states in southwestern Nigeria .Serum was prepared from the blood samples. Elisa kit was used for the experiment and micro-titer plate reader was used to read the result with the absorbance wave length of 450nm.Thirteen samples were observed positives [48.1%] and fourteen samples were observed negatives [58.1%].From this result it was concluded that less percentage of the population studied had anti-Dengue virus anti IgM antibodies.

CHAPTER 1

1.0 INTRODUCTION

Key word: Dengue, virus, antibodies, malaria, typhoid, patients, clinic.

Dengue virus has been classified in past years as one of the Family of flaviridae, Genus of flavivirus Species name is Dengue virus[*Modis et al.*, 2004] And is one of the most important vector borne disease of modern man with about 50 million of infected people per year [domingo et al.,2006] dengue is an acute viral illness characterized by fever, muscular pan ,rash ,headache and some thrombocytopoaenia .more severe form of dengue infection include :dengue hemorrhagic fever and dengue shock syndrome with both form having a high mortality rate [Garg 2011] Dengue virus has a antigenically distant serotype ,type 1 to 4 with each serotype capable of causing severe disease and infection with one of these serotype provide lifelong immunity to infecting serotype only .The principal vector of this virus is *Aedes aegypti* others include *Aedes alboptious*[Garg 2011]

In Africa dengue virus was first isolated in Ibadan Nigeria around 1960 [Amarasingle et al., 2011] there have been several report of isolated outbreak of dengue infection till date.[gunther et al,. 2007]The early symptom of arbovirus infection [high grade fever ,headache, fatigue nausea, vomiting, mimic-malaria ,typhoid, measles and influenza which are hyper endemic in the environment thereby rendering the diagnosis of this viral infection very confusing in such situation these infection are quite often misdiagnosis and so inappropriately treated and consequently these case often result in high rate of mobility complication and mortality .yet health institution in Nigeria lack appropriate diagnosis facilities for this group of virus even with the existence of factors [human population ,increased urbanization ,increasing human activity into the new ecosystem ,increase global travel ,climate changes and collapse of vector control and public health program[Gubler 2007] which

favors the emergence of dengue globally .the diagnosis of dengue infection require laboratory confirmation ,either by isolating the virus or detecting dengue specific antibodies for virus .isolation or detection of dengue RNA in serum specimen by serotype specific, real time reverse transcriptase polymerase chain reaction [RT-PCR],an acute phase serum specimen should be collected within 5 day of symptom onset.

In Nigeria ,malaria and typhoid is generally ascribe to all febrile illness unless confirmed through laboratory testing ,febrile patient regularly exhibit symptom thought to be caused by malaria or typhoid but which are also commonly observed with dengue virus infection. Malaria, typhoid and dengue are diseases that have significant worldwide mortality and morbidity. It is estimated that more than 100 million people are infected with typhoid, causing more than a million deaths per year, and 50–100 million people are infected with dengue, including 500,000 cases of dengue hemorrhagic fever per year. Malarial is caused by obligate intracellular parasite which live in host erythrocyte and remodel these cell to provide optimally for their own need .it is a major public health problem in tropic area and is estimated that malaria is responsible for 1 to3 million death 300 to 500 million infection annually .on the other hand typhoid fever is widely recognized as a major public health problem in most developing tropical countries [Pearson et al., 2000]

AIM

To detect the prevalence of dengue virus IgM among patient attending clinic for typhoid and malaria in different states

The objectives of this research project are:

1. To detect dengue virus antibodes IgM from patient attending clinic for typhoid and malaria fever

2. To establish possible differential diagnosis of dengue infection in malaria and typhoid patients

3.To improve dengue surveillance and diagnostic so that the quality of care for patient can be improve and the cost and rate associated with malaria ,typhoid and dengue infection could be reduce.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HISTORY

The first record of a case of probable dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265–420 AD) which referred to a "water poison" associated with flying insects. The primary vector, *A. aegypti*, spread out of Africa in the 15th to 19th centuries due in part to increased globalization secondary to the slave trade. There have been descriptions of epidemics in the 17th century, but the most plausible early reports of dengue epidemics are from 1779 and 1780, when an epidemic swept across Asia, Africa and North America. From that time until 1940, epidemics were infrequent. (Gubler, 2002).In 1906, transmission by the *Aedes* mosquitoes was confirmed, and in 1907 dengue was the second disease (after yellow fever) that was shown to be caused by a virus. Further investigations by John Burton Cleland and Joseph Franklin Siler completed the basic understanding of dengue transmission. The marked spread of dengue during and after the Second World War has been attributed to ecologic disruption. The same trends also led to the spread of different serotypes of the disease to new areas, and to the emergence of dengue hemorrhagic fever. This severe form of the disease was first reported in the Philippines in 1953; by the 1970s, it had become a major cause of child mortality and had emerged in the Pacific and the Americas. Dengue hemorrhagic fever and dengue shock syndrome were first noted in Central and South America in 1981, as DENV-2 was contracted by people who had previously been infected with DENV-1 several years earlier

Dengue is the most common arthropod-borne viral infection in the world (Gubler, 2006). The disease is endemic in more than 100 countries throughout Africa, the Americas, the Eastern Mediterranean, South-East Asia, and the Western Pacific (Normile,et,al 2013). There are four distinct serotypes of dengue virus (DENV) and each of these serotypes can cause disease symptoms ranging from self-limited febrile illness called dengue fever (DF) to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Martina, 2009). Infection with one serotype confers protective immunity against that serotype but not against other serotype (Halstead, 2008). In fact, several retrospective and prospective studies have revealed that secondary infection with a heterologous serotype is a risk factor for developing DHF/DSS (Scott, 2008). Also, infants born to dengue immune mothers are at risk to develop more severe dengue during a primary infection (Reiter, 2010). This suggests that antibodies play an important role in controlling the outcome of an infection. It is believed that antibodies specifically direct the virus particles to cells carrying Fc-receptors (FcR), such as monocyte, macrophages, and dendritic cells, which—as the natural targets for the virus—are permissive for DENV infection (Reiter, 2010). This leads to enhanced infection of these cells and thus, to high viral loads, resulting in extensive T cell activation early in the infection process. As a consequence, high amounts of cytokines and chemical mediators are released, which may lead to endothelial cell damage and subsequent plasma leakage (Whitehorn, 2010). Other factors that are implicated in disease pathogenesis include viral virulence, the ethnic background and age of the individual and specific epidemiological conditions (Bhatt, 2013). This review will give a general overview of the infectious life cycle of DENV,

and describe the viral and host factors that may influence disease outcome (Harris *et al*,1998).

In the absence of a vaccine or any specific drug for its treatment, an early diagnosis is considered indispensable to prevent any casualty. Detection of viruses in human sera particularly in endemic areas is cumbersome, difficult and also not desirable. Therefore, as an alternative approach, detection of the dengue virus antigen in mosquitoes has provided a reliable tool to (*i*) comprehend the types of viruses circulating in nature; and (*ii*) help in designing vector-specific control strategies. A mélange of diagnostic techniques are currently available with some advantages or disadvantages. Traditionally, cell cultures and suckling mice have been employed for virus isolations. While the virus isolation method in baby mice is time consuming, slow and expensive, the mosquito cell cultures offer a good degree of specificity (Letson,et al2011). Mosquito inoculation techniques have been reported for detection and propagation of flavi viruses. Though this technique is sensitive for routine virological confirmation of dengue fever, it requires large number of infected mosquitoes, besides being time consuming. Insect bioassays (Toxo-IFA) are generally cumbersome requiring special facilities and are not suitable for large-scale epidemiological surveillance. ELISA has been shown to be a rapid and sensitive alternative to insect bioassays for monitoring arboviruses in wild populations. Reverse transcriptase polymerase chain reaction (RT-PCR) is a recent molecular diagnostic technology used for detecting virus infections in mosquitoes, which gives rapid results but is expensive and prone to contamination.

Definite diagnosis of the impending dengue epidemic can be made using ELISA for virological surveillance system on dengue virus antigen in the mosquito

vectors. Therefore, ELISA offers a potential tool and a convenient system for quickly screening large number of samples up to the serotype level which can be employed effectively and efficiently for large scale dengue surveillance programs on wild caught mosquito vectors. ELISA positive samples can be screened further by Toxo-IFA system for virus isolation. On the other hand, techniques like mosquitoes cell culture, mosquito inoculation (Toxo-IFA) and RT-PCR techniques can be employed for dengue virus amplification. Dengue virus (DENV) is an RNA virus of the family *Flaviviridae*; genus *Flavivirus*. Other members of the same genus include yellow fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kyasanur forest disease virus, and Omsk hemorrhagic fever virus· Most are transmitted by arthropods (mosquitoes or ticks), and are therefore also referred to as arboviruses (*ar*thropod-*bo*rne viruses).The dengue virus genome (genetic material) contains about 11,000 nucleotide bases, which code for the three different types of protein molecules that form the virus particle and seven other types of protein molecules that are only found in infected host cells and are required for replication of the virus. There are five strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. The fifth type was announced in 2013.The distinctions between the serotypes are based on their antigencity.

2.2 TRANSMISSION

The mosquito *Aedes aegypti* feeds on a human host. Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*. These mosquitoes usually live between the latitudes of 35° North and 35° South below an elevation of 1,000 meters (3,300 ft)(WHO 2009) They typically bite during the day, particularly in the early morning and in the evening, but they are able to bite and thus spread infection at any time of day all during the year. Other *Aedes* species that transmit the disease include *A. albopictus*, *A. polynesiensis* and *A. scutellaris*. Humans are the primary host of the virus, but it also circulates in nonhuman primates .An infection can be acquired via a single bite.A female mosquito that takes a blood meal from a person infected with dengue fever, during the initial 2–10 day febrile period, becomes itself infected with the virus in the cells lining its gut (Johnson, 2009). About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life.*Aedes aegypti* is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates.(Gubler,et al 2010).

Dengue can also be transmitted via infected blood products and through organ donation. In countries such as Singapore, where dengue is endemic; the risk is estimated to be between 1.6 and 6 per 10,000 transfusions. Vertical transmission (from mother to child) during pregnancy or at birth has been reported. Other person-to-person modes of transmission have also been reported, but are very unusual.(Chen et al, 2010).The genetic

variation in dengue viruses is region specific; suggestive that establishment into new territories is relatively infrequent, despite dengue emerging in new regions in recent decades (Barret, 2009)

2.3 PREDISPOSITION

Severe dengue virus is more common in babies and young children, and in contrast to many other infections it is more common in children that are relatively well nourished(Tony, 2010).Other risk factors for severe disease include female sex, high body mass index, and viral load. While each serotype can cause the full spectrum of disease, virus strain is a risk factor. Infection (WHO, 2013) with one serotype is thought to produce lifelong immunity to that type, but only short-term protection against the other three. The risk of severe disease from secondary infection increases if someone previously exposed to serotype DENV-1 contracts serotype DENV-2 or DENV-3, or if someone previously exposed to DENV-3 acquires DENV-2.Dengue (Bridget, 2013) can be life-threatening in people with chronic diseases such as diabetes and asthma(Kissoon,2011).

Polymorphisms (normal variations) in particular genes have been linked with an increased risk of severe dengue complications. Examples include the genes coding for the proteins known and particular forms of human leukocyte antigen from gene variations (Guzman et al., 2010). A common genetic abnormality, especially in Africans, known as glucose-6-phosphate dehydrogenase deficiency, appears to increase the risk. Polymorphisms in the genes for the vitamin D receptor and FcγR seem to offer protection against severe disease in secondary dengue infection.

2.4 VIRAL MECHANISM

When a mosquito carrying dengue virus bites a person, the virus enters the skin together with the mosquito's saliva. It binds to and enters white blood cells, and reproduces inside the cells while they move throughout the body. The white blood cells respond by producing a number of signaling proteins, such as cytokines and interferon, which are responsible for many of the symptoms, such as the fever, the flu-like symptoms and the severe pains. In severe infection, the virus production inside the body is greatly increased, and many more organs (such as the liver and the bone marrow) can be affected. Fluid from the bloodstream leaks through the wall of small blood vessels into body cavities due to capillary permeability. 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. Furthermore, dysfunction of the bone marrow due to infection of the stromal cells leads to reduced numbers of platelets, which are necessary for effective blood clotting; this increases the risk of bleeding, the other major complication of dengue fever (Stanberry,2009).

2.5VIRAL REPLICATION

Once inside the skin, dengue virus binds to Langerhans cells (a population of dendritic cells in the skin that identifies pathogens). (Martina et al., 2009). The virus enters the cells through binding between viral proteins and membrane proteins on the Langerhans cell, specifically the C-type lectins, mannose receptor a non-specific receptor for foreign material on dendritic cells, seems to be the main point of

entry.(Guzman *et al*, 2010). The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins that replicate the viral RNA and begin to form viral particles. Immature virus particles are transported to the Golgi apparatus, the part of the cell where some of the proteins receive necessary sugar chains glycoproteins. The now mature new viruses bud on the surface of the infected cell and are released by exocytosis. They are then able to enter other white blood cells, such as monocytes and macrophages (Stramer et al., 2009).

The initial reaction of infected cells is to produce interferon, a cytokine that raises a number of defenses against viral infection through the innate immune system by augmenting the production of a large group of proteins mediated by the JAK-STAT pathway. Some serotypes of dengue virus appear to have mechanisms to slow down this process. Interferon also activates the adaptive immune system, which leads to the generation of antibodies against the virus as well as T cells that directly attack any cell infected with the virus. Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis (ingestion by specialized cells and destruction), but some bind the virus less well and appear instead to deliver the virus into a part of the phagocytes where it is not destroyed but is able to replicate further (Sampath et al., 2010).

2.6 DIAGONSIS

Warning signs

Worsening abdominal pain

Ongoing vomiting

Liver enlargement

Mucosal bleeding

High hematocrit with low platelet

Lethargy or restlessness

Serosal effusions

The diagnosis of dengue is typically made clinically, on the basis of reported symptoms and physical examination; this applies especially in endemic areas. (Whitehorn et al., 2010).However, early disease can be difficult to differentiate from other viral infections. A probable diagnosis is based on the findings of fever plus two of the following: nausea and vomiting, rash, generalized pains, low white blood cell count, positive tourniquet test, or any warning sign in someone who lives in an endemic area. (WHO 2009). Warning signs typically occur before the onset of severe dengue. (WHO, 2009). The tourniquet test, which is particularly useful in settings where no laboratory investigations are readily available, involves the application of a blood pressure cuff at

between the diastolic and systolic pressure for five minutes, followed by the counting of any petechial hemorrhages; a higher number makes a diagnosis of dengue more likely with the cut off being more than 10 to 20 per 1 inch^2 (6.25 cm2) (Tomlinson, 2009).

The diagnosis should be considered in anyone who develops a fever within two weeks of being in the tropics or subtropics. It can be difficult to distinguish dengue fever and chikungunya, a similar viral infection that shares many symptoms and occurs in similar parts of the world to dengue. Often investigations are performed to exclude other conditions that cause similar symptoms, such as malaria, leptospirosis, viral hemorrhagic fever, typhoid fever, meningococcal disease, measles, and influenza (Halasa,et al., 2013).The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets and metabolic acidosis. A moderately elevated level of amino transferase (AST and ALT) from the liver is commonly associated with low platelets and white blood cells. In severe disease, plasma leakage results in hemo-concentration (as indicated by a rising hematocrit) and hypo-albuminemia. Pleural effusions or ascites can be detected by physical examination when large, but the demonstration of fluid on ultra sound may assist in the early identification of dengue shock syndrome. The use of ultrasound is limited by lack of availability in many settings. Dengue shock syndrome is present if pulse pressure drops to ≤ 20 mm Hg along with peripheral vascular collapse. Peripheral vascular collapse is determined in children via delayed capillary refill, rapid heart rate, or cold extremities. [WHO,2009).While warning signs are an important aspect for early detection of potential serious disease, the evidence for any specific clinical or laboratory marker is weak. (WHO, 2002).

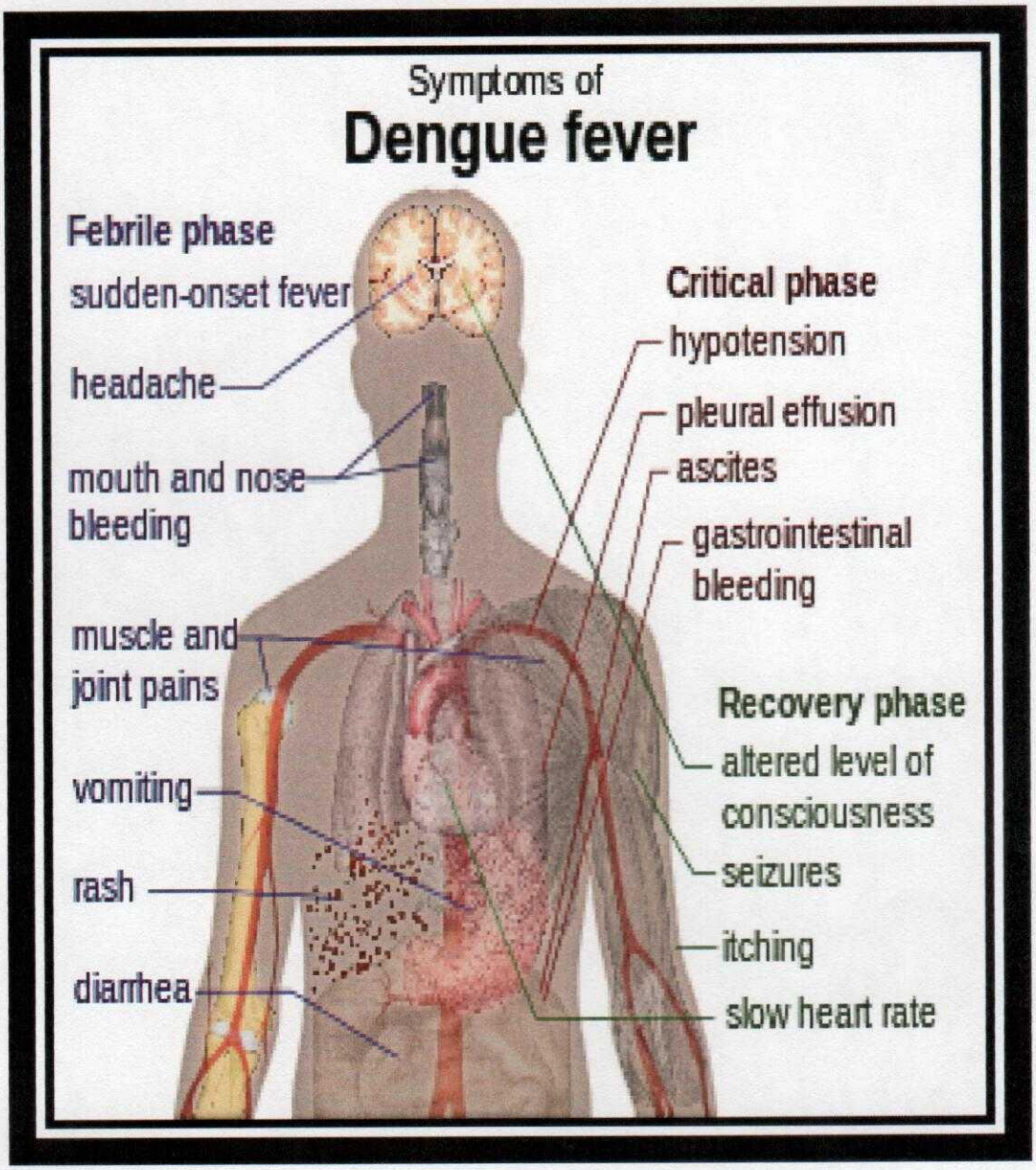

PLATE 1: SYMPTOMS OF DENGUE FEVER

2.7CLASSIFICATION

The World Health Organization's 2009 classification divides dengue fever into two groups: uncomplicated and severe. This replaces the 1997 WHO classification, which needed to be simplified as it had been found to be too restrictive, though the older classification is still widely used including by the World Health Organization's Regional Office for South-East Asia as of 2011.Severe dengue is defined as that associated with severe bleeding, severe organ dysfunction, or severe plasma leakage while all other cases are uncomplicated. The 1997 classification divided dengue into undifferentiated fever, dengue fever, and dengue hemorrhagic fever. Dengue hemorrhagic fever was subdivided further into grades I–IV. Grade I is the presence only of easy bruising or a positive tourniquet test in someone with fever, grade II is the presence of spontaneous bleeding into the skin and elsewhere, grade III is the clinical evidence of shock, and grade IV is shock so severe that blood pressure and pulse cannot be detected. Grades III and IV are referred to as "dengue shock syndrome".

The diagnosis of dengue fever may be confirmed by microbiological laboratory testing. This can be done by virus isolation in cell cultures, nucleic acid detection by PCR, viral antigen detection (such as for NS1) or specific antibodies (serology).Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost. Detection of NS1 during the febrile phase of a primary infection may be greater than 90% sensitive however only 60–80% in subsequent infections is. All tests may be negative in the early stages of the disease. PCR and viral antigen detection are more accurate in the first seven days. In 2012 a PCR test was

introduced that can run on equipment used to diagnose influenza; this is likely to improve access to PCR-based diagnosis (Wolff, 2009).

These laboratory tests are only of diagnostic value during the acute phase of the illness with the exception of serology. Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5–7 days. The highest levels (titres) of IgM are detected following a primary infection, but IgM is also produced in re-infection. IgM becomes undetectable 30–90 days after a primary infection, but earlier following re-infections. IgG, by contrast, remains detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection. After a primary infection IgG reaches peak levels in the blood after 14–21 days. In subsequent re-infections, levels peak earlier and the titres are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus. In testing for IgG and IgM antibodies there may be cross-reactivity with other flaviviruses which may result in a false positive after recent infections or vaccinations with yellow fever virus or Japanese encephalitis. The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic (Thurman et al., 2010).

2.8PREVENTION

There are no approved vaccines for the dengue virus. Prevention thus depends on control of and protection from the bites of the mosquito that transmits it. The World Health Organization recommends an Integrated Vector Control program consisting of five elements:

1. Advocacy, social mobilization and legislation to ensure that public health bodies and communities are strengthened;
2. Collaboration between the health and other sectors (public and private);
3. An integrated approach to disease control to maximize use of resources;
4. Evidence-based decision making to ensure any interventions are targeted appropriately; and
5. Capacity-building to ensure an adequate response to the local situation.

The primary method of controlling *A. aegypti* is by eliminating its habitats. This is done by getting rid of open sources of water, or if this is not possible, by adding insecticides or biological control agents to these areas. Generalized spraying with organophosphate or pyrethroid insecticides, while sometimes done, is not thought to be effective. Reducing open collections of water through environmental modification is the preferred method of control, given the concerns of negative health effects from insecticides and greater logistical difficulties with control agents. People can prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while resting, and/or the application of insect repellent (DEET being the most effective).However, these methods appear not to be sufficiently effective, as the frequency of outbreaks appears to be

increasing in some areas, probably due to urbanization increasing the habitat of *A. aegypti*. The range of the disease appears to be expanding possibly due to climate change (Wilson et al., 2002]

2.9EPIDEMOLOGY

Most people with dengue recover without any ongoing problems. The fatality rate is 1–5%, and less than 1% with adequate treatment; however those who develop significantly low blood pressure may have a fatality rate of up to 26%.Dengue is common in more than 110 countries. It infects 50 to 528 million people worldwide a year, leading to half a million hospitalizations and approximately 25,000 deaths (Gould et al, 2009).For the decade of the 2000s, 12 countries in Southeast Asia were estimated to have about 3 million infections and 6,000 deaths annually (Robaina,2003). It is reported in at least 22 countries in Africa; but is likely present in all of them with 20% of the population at risk. This makes it one of the most common vector-borne diseases worldwide.(Yacoub *et al*, 2014).Infections are most commonly acquired in the urban environment. In recent decades, the expansion of villages, towns and cities in the areas in which it is common, and the increased mobility of people have increased the number of epidemics and circulating viruses (Delgoda et al., 2007).

Dengue fever, which was once confined to Southeast Asia, has now spread to Southern China, countries in the Pacific Ocean and America, and might pose a threat to Europe (Gomez, 2005).Rates of dengue increased 30 fold between 1960 and 2010.This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming. The geographical distribution is around the

equator. Of the 2.5 billion people living in areas where it is common 70% are from Asia and the Pacific. An infection with dengue is second only to malaria as a diagnosed cause of fever among travelers returning from the developing world. It is the most common viral disease transmitted by arthropods, (Rodenhuis *et al*,2010) has a disease burden estimated at 1,600 disability-adjusted life years per million population.(Guzman, Halstead, Artsob, et al., 2010). The World Health Organization counts dengue as one of seventeen neglected tropical diseases (WHO, 2013) like most arboviruses, dengue virus is maintained in nature in cycles that involve preferred blood-sucking vectors and vertebrate hosts. The (Vaughan and Kuno, 2001) viruses are maintained in the forests of Southeast Asia and Africa by transmission from female *Aedes* mosquitoes—of species other than (Devine, 2005)*A. aegypti*—to their offspring and to lower primates. In towns and cities, the virus is primarily transmitted by the highly domesticated *A. aegypti*. In rural settings the virus is transmitted to humans by *A. aegypti* and other species of *Aedes* such as *A. albopictus*. Both these species had expanding ranges in the second half of the 20th century. In all settings the infected lower primates or humans greatly increase the number of circulating dengue viruses, in a process called amplification (Ruben et al., 2000).

2.9.1 TREATMENT AND MANAGEMENT OF DENGUE FEVER

There is no vaccine or specific treatment available against dengue infection. It is difficult to develop a vaccine because of the 4 different dengue viruses that cause the disease. There is no specific anti-viral tablet or injection that can kill the dengue virus,

but a lot of treatment can help to save a patient suffering from dengue fever. The fever is treated by anti-pyretic like: paracetamol, the pain in the bone can be treated by analogesics or pain killing tablet. In the case of patient suffering from dengue hemorrhagic fever or dengue shock syndrome then hospitalization is a must .patient are prone to dehydration hence they should drink plenty of fluid. Because dengue is a virus there is no specific treatment or cure, however there are things the patient or the doctor can do to help, depending on the severity of the disease. For milder forms of dengue the treatment methods are: Blood transfusion - a blood transfusion may be recommended for patients with severe dehydration. Hospital care - it is important that you be treated by medical professionals, this way you can be properly monitored (e.g. fluid levels, blood pressure) in case your symptoms worsen. If the patient is cared for by physicians and nurses experienced with the effects and complications of hemorrhagic fever, lives can be saved. The most important aspect in treatment of DHF is to prevent further fluid loss. Drugs such as corticosteroids or carbazochrome sodium sulfonates are given to stabilize capillary permeability and avoid plasma leakage. As regards to prevention, a vaccine has proven rather difficult to produce. The reason for this stems from the 4 subtypes of dengue. If an individual develops immunity to one subtype and then tries to launch an immune response to another subtype then they will develop DHF/DSS. Work has been done on a tetravalent vaccine that will attempt to give the individual immunity to all four of the subtypes at the same time. Currently, the most effective prevention measures lie in mosquito control.

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1 SAMPLE COLLECTION

A total of 27 blood samples of fresh blood was collected from patients (children and adults) reporting for malaria and typhoid from different states. The blood was intravenously collected using a 5ml syringe. A tourniquet was tied on the arm of patient, cotton wool dip inside ethanol was used to swab the arm, and 2-3ml of blood was collected from each patient from both male and female young adults between the ages (1-67 years) and children. The collected blood samples were poured inside bottles to allow clotting leaving it undisturbed at room temperature. This takes 10 -20minutes. Each bottle was labeled according to their age, sex and location respectively.[male:9 and female;17]

3.1.1 STORAGE OF SAMPLES

The collected blood samples in the bottle were spin at 2000 – 3000 rpm for 20minutes and the blood was separated from the serum, putting the serum into a different bottle labeled and stored in the freezer at temperature of -20^{o}c.

3.1.2 MATERIAL, EQUIPMENTS AND REAGENT

Conical flask, micropipette, micropipette tips, warm bath shaker, micro titer plate reader, human dengue virus IgM ELISA kit contain: closure plate membrane, micro Elisa strip plate, negative control, positive control, HRP conjugate reagent, sealed bags, sample diluents, chromogen solution A, chromogen B, stop solution, wash solution.

3.2KIT PRECAUTION

- The kit should be keep at 4°C upon receipt. The kit should be equilibrat4ed to room temperature before the assay.
- Any unneeded strips should be removed from DV antigen –Coated plate, they should be resealed in zip lock foil and kept at 4°c.
- The precipitate appears in concentrated washing buffer. According to manufacturer instruction, it should be heated to dissolve all the precipitates, which will not affect the result
- In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
- The substrate should be kept away from light.
- All the operation must be accordance with the manufacturer's instructions strictly. The results determined by the Micro Elisa strip plate Reader.
- All the samples, washing buffer and wastes were treated as infectious agents.
- Reagents from different lots should not be mixed.

3.3 PROCEDURES

In the Micro Elisa strip plate, two wells should be left as negative control, two wells as positive control and one well empty as blank control. The sequential number should follow, corresponding sample of the micro porous hole. 2 per board were set negative control and positive control 2 holes, ck 1 hole, Samples was be added under negative and positive control in a volume of 50µl to the negative and positive control

wells respectively. In sample wells, 40µl Sample dilution buffer and 10µl sample were added. Samples were loaded on to the bottom without touching the well wall. The well was mixed with gentle shaking. The micro Elisa strip plate was incubated after been sealed with closure plate membrane for 30minutes at 37°c. The concentrated washing buffer was diluted with distilled water. The closure plate membrane was carefully peeled off, aspirated, refilled with wash solution. The wash solution was discarded after resting for 30seconds. The washing solution was repeated 5times. Into every well except the blank well, 50µl HRP conjugate reagent was added. Incubation was done for 30minutes at 37°c and washing is done repeatedly just like the first washing for 5times. The addition of 50 µl of chromagen A and chromagen B follows and was gently incubated by shaking for 37°c for 15minutes. Addition of 50µl of stop solution to terminate the reaction of each well. The well must change from blue to yellow. The micro strip plate was read at 450wavelength using micro titer plate reader. The blank plate was set to zero. The assay was read within 15minutes after dropping the stop solution.

3.4 THE PRINCPLE OF THE KIT.

This ELISA kit uses Sandwich-ELISA as the method. The Micro-elisa strip plate provided in the kit has been pre-coated with an antigen specific to DV-IgM. Standards or samples are added to the appropriate Micro-elisa strip plate wells and combined to the specific antigen. Then a Horseradish Peroxidase (HRP)-conjugated antigen specific for DV is added to each Micro-elisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain DV-IgM and HRP conjugated DV antigen will appear blue in

color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wave length of 450 nm. The presence of DV-IgM is determined by comparing with the CUT OFF value.

3.5 PRECAUTION

• This kit is for research use only.

• Please use only the valid version of the package insert provided with the kit.

• All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIVI/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

• Controls and Standards have been found to be non-infectious in cell cultures.

• Avoid contact with Stop Solution containing 0.2mol/L H2SO4. It may cause skin irritation and burns.

• Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.

• Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

• Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.

• Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.

• Do not use reagents beyond expiry date as shown on the kit labels.

• All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and micro titer plate readers.

• Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.

• Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.

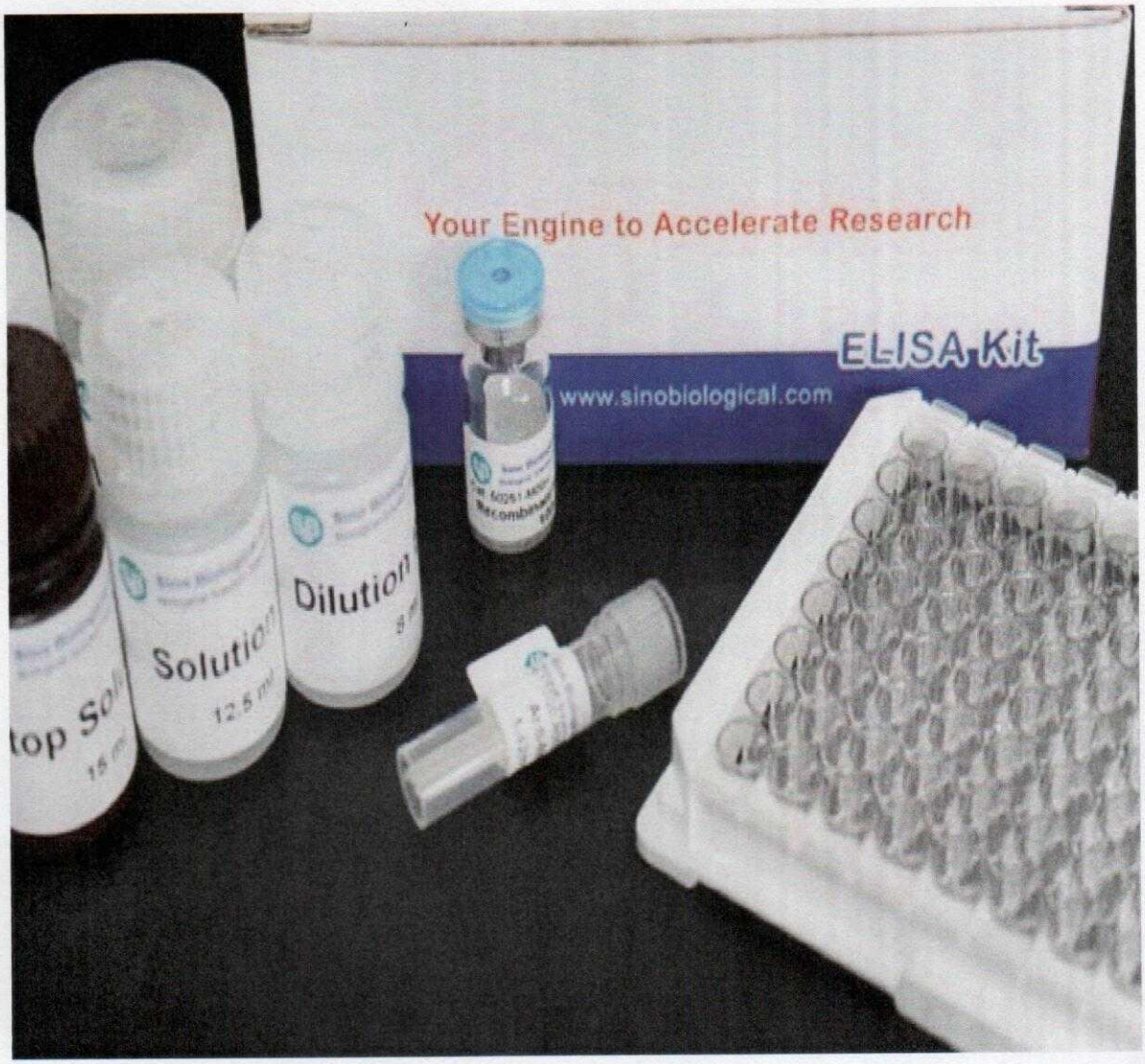

PLATE 1: ELISA KIT AND REAGENTS

PLATE 2: MICROTITER PLATE READER

CHAPTER FOUR

4.0 RESULT

After all detection of IgM in blood samples, ELISA was performed showing the result positive for 13samples and negative for 14 samples of the blood.

Table 1: Result of blood samples containing the age, sex and location.

AGE	SEX				LOCATION
	MALE +TIVE	MALE -TIVE	FEMALE +TIVE	FEMALE -TIVE	
1-10	1				Ado-ekiti
			1		Akure
				1	Akure
11-20			1		Jabu (Lagos)
			1		Jabu (Lagos)
				2	Ado –Ekiti
21 – 30			1		Ikeji
				1	Akure
				1	Jabu(Lagos)
				3	Ikeja
			1		Ado –Ekiti
	1				Ado –Ekiti
		1			Akure

			1		Ikeji
		1			Ado –Ekiti
		1			Akure
		1			Ikeji
31-40	1				Ikeji
			1		Akure
				1	Akure
41 -50	1				Ikeji
51 -60	1		1		Ikeji
					Ikeji
61 -70		1			Ado –Ekiti

Table 2: Comparison of viral prevalence between male and female subjects

SEX	NUMBER OF SAMPLES	NO OF NEGATIVE	NO. OF POSITVE	POSITIVE %	NEGATIVE %
MALE	9	5	5	66.6	44.4
FEMALE	17	9	8	47.1	47.1
TOTAL	26	14	13	42%	58%

From the result it was shown that dengue virus with IgM ELISA test was 48.1% negative and 51.8% positive in all the blood samples tested.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMEDATION

5.1CONCLUSION

The percentage of negative result obtained in this project work interprets that less percentage of dengue virus in this population studied had anti-dengue virus anti IgM antibodies

5.2 RECOMMENDATION

. I recommend that people should always treat themselves when they notice the symptoms in them before it get hard for treatment and because the symptom of dengue, malaria and typhoid are the same it is important that proper diagnosis of the disease should be done .Patients with Dengue fever can have poor immunity during convalescent period which makes them susceptible to other infections. Beyond one week of fever, if patient with Dengue infection shows no signs of response to conservative treatment, it is strongly suggested to consider other possibilities of co infections most importantly Bacterial infections and Malaria. Incidence is low in the area tested

REFERENCES

Ananda Amarasinghe, Joel N. Kuritsky, G. William Letson, and Harold S. Margolis [2011] *J of dengue virus infection in Africa vol 17 ,8-10*

Allele frequencies in Cuban individuals with antecedents of dengue 2 disease: advantages of the Cuban population for HLA studies of dengue virus infection. Human Immunology ` 68:531– 540

Allison SL, Stadler K, Mandl CW, Kunz C, Heinz FX (1995) Synthesis and secretion of recombinant tick-borne encephalitis virus protein E in soluble and particulate form. *Journal Virology* 69:5816–5820

Bryant JE, Calvert AE, Mesesan K, Crabtree MB, Volpe KE,Silengo S, Kinney RM, Huang CY, Miller BR, Roehrig JT(2007) Glycosylation of the dengue 2 virus E protein at N67 is critical for virus growth in vitro but not for growth in intra-thoracically inoculated Aedes aegypti mosquitoes. *Journal Virology* 366:415–423

Burke DS, Monath TP (2001) Flaviviruses. In: Knipe DM, Howley PM (eds) Fields virology. Lippincott Williams and Wilkins, Philadelphia, pp 1043–1125

Burke DS, Nisalak A, Johnson DE, Scott RM (1988) A pro- spective study of dengue infections in Bangkok. *Journal of Tropical Medicine, volume 38:172*–180

Chang CJ, Luh HW, Wang SH, Lin HJ, Lee SC, Hu ST (2001) The heterogeneous nuclear ribo nucleoprotein K interacts with dengue virus core protein. DNA Cell Biology 20:569–577

Clyde K, Kyle JL, Harris E (2006) Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *Journals Virology* 80:11418–11431277:23–39

Crabtree MB, Kinney RM, Miller BR (2005) De-glycosylation of the NS1 protein of dengue 2 virus, strain 16681: construction and characterization of mutant viruses. Arch Virology 150:771–786

Flamand M, Megret F, Mathieu M, Lepault J, Rey FA, Deubel V (1999) Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble hexamer in a gly- cosylation-dependent fashion. *Journal of Virology* 73:6104–6110

Fonseca BA, Pincus S, Shope RE, Paoletti E, Mason PW (1994) Recombinant vaccinia viruses co-expressing dengue-1 glyco- proteins prM and E induce neutralizing antibodies in mice. Vaccine 12:279–285

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

Gubler DJ (2006) Dengue/dengue haemorrhagic fever: history and current status. Molecular biology of flavi viruses. Novartis Found Symp,277:3–16

Gubler DJ,Sather GE,Kuno G, Cabral JR.[2011] Dengue 3 Virus transmission in Africa.*virol. Journal of public health,vo [6] ;1280-1284.*

Guzman MG, Kouri G, Bravo J, Valdes L, Vazquez S, Halstead SB (2002) Effect of age on outcome of secondary dengue 2 infections. *International Journal of Infect Disease 6:118–124*

Guzman MG, Kouri G, Valdes L, Bravo J, Vazquez S, Halstead SB (2002) Enhanced severity of secondary dengue-2 infections: death rates in 1981 and 1997 Cuban outbreaks. Revised Panam Salud Publication 11:223–227

Halstead SB (1970) Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med 42:350–362

Halstead SB, Lan NT, Myint TT, Shwe TN, Nisalak A, Kaly- anarooj S, Nimmannitya S, Soegijanto S, Vaughn DW, Endy TP (2002) Dengue hemorrhagic fever in infants: research opportunities ignored. Emergence Infected Disease 8:1474–1479

Hunt AR, Cropp CB, Chang GJ (2001) A recombinant particulate antigen of Japanese encephalitis virus produced in stably-transformed cells is an effective noninfectious antigen and subunit immunogen. *Journal of Virology* Methods 97:133–149

Jessie K, Fong MY, Devi S, Lam SK, Wong KT (2004) Localization of dengue virus in naturally infected human tissues, by immunehistochemistry and in situ hybridization. *Journal of Infectious Disease* 20 112-125

Jones CT, Ma L, Burgner JW, Groesch TD, Post CB, Kuhn RJ (2003) Flavivirus capsid is a dimeric alpha-helical protein. *J of Virol* 77:7143–7149189:1411–1418

Kliks SC, Nimmanitya S, Nisalak A, Burke DS (1988) Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. Am *J Trop Med Hyg 38:411–419*