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Serofrequency of Dengue Virus Infection among Febrile Patients in Eastern Sudan

التكرار المصلي لعدوى فيروس حمى الضنك وسط المرضى المصابين بالحمى في شرق السودان

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DEDICATION

To my Parents, who always give me love and care….

To those who help me more, my Teachers…….

To those whom I love more, and my Friends…….

To all people who love me

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I take this opportunity to express my sincere, heartfelt gratitude to Almighty ALLAH for giving me the health and wealth to make this research a reality. Exquisite thanks go to my supervisor, **Dr. Hind Haidar Ahmed,** for her unrest guidance, wisdom and advice couple with patience. Also, my appreciation goes to Sudan University of Science and Technology. Special gratitude to **Prof. Moawia Mokhtarand Dr. Mona Omer,** Institute of Endemic Disease for their technical support.

ABSTRACT

Dengue fever, caused by dengue virus (DENV), which has become one of the most important mosquitos borne viral diseases with a steady rise in global incidence, including the Sudan. Sporadic cases and frequent acute febrile illness outbreaks, compatible with Dengue fever, have been reported in Eastern Sudan especially Port Sudan and Kassala State. This descriptive cross-sectional study was aimed to determine the frequency and potential risk factors of dengue virus infection in Eastern Sudan at Kassala and Port Sudan Teaching Hospitals between December 2019 and April 2022.

In this study 93 blood specimens wererandomly collected from febrile patients and they tested for the presence of DENV-specific immunoglobulin (IgM) antibodies using a commercially available Anti-dengue IgM enzyme-linked immunosorbent assay (ELISA).

Among the 93 febrile patients, 83 (89.2%) were seropositive for DENV IgM, while 10(10.8%) were negative. Concerning positivity according to the age groups, there were 36 (43.3%) in the age group 1-5 years, 32 (38.6%) in the age group 6-11 years, and 15(16.1%) at age groups 12-17 years were positive for anti-DENV IgM antibodies. There was an in significant association between age group and Dengue fever (*P*-value = 0.075). For gender, 42 (50.6%) male, 41 (49.4%) females were positive for the presence of anti-DEVN IgM antibodies, but there was no meaningful association between gender and Dengue fever infection (*P*.value=0.97).On average, 74 (89.2%) of the 83 patients with anti-DENV antibodies had symptoms lasting from 3-7 days, while 9 patients (9.8%) had symptoms lasting more than 7 days. An insignificant association was found between anti-DENV-antibodies and symptoms' duration (*P*.value=0.65).Among the positive patients, 22 (26.5%) were from Port Sudan, while 61 (74.5%) were from Kassala. The residence group was not significantly associated with anti-DENV antibodies (*P*.value =0.23).

In conclusion: There was a high frequency of DENV antibodies among febrile patients in Eastern Sudan, because during that time Red Sea and Kassala States experiencing epidemics of fever.

المستخلص

أصبحت حمى الضنك، التي يسببها فيروس حمى الضنك ، واحدة من أهم الأمراض الفيروسية التي ينقلها البعوض مع ارتفاع مطرد في الإصابة العالمية، بما في ذلك السودان. تم الإبلاغ عن حالات متفرقة وحالات متكررة لأمراض الحمى الحادة المتوافقة مع حمى الضنك في شرق السودان خاصة بورتسودان وكسلا. هذه دراسة وصفية مقطعية هدفتلدراسة انتشار الأجسام المضادة لـ حمى الضنك ، وتحديد عوامل الخطر المحتملة المرتبطة بهذا المرض بين المقيمين في شرق السودان في مستشفي بورتسودان و كسلا التعليميي في الفترة بين ديسمبر2019 الي ابريل 2022.

في هذة الدراسة تم جمع 93 عينة دم من المرضى المصابين بالحمى وتم اختيارهم عشوائياً من شرق السودان وخاصة ولاية بورتسودان وكسلا. تم اختبار الأمصال من 93 مريضا بالحمي لوجود أجسام مضادة خاصة (IgM) بحمى الضنك وتحديد عوامل الخطر باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA).

من ضمن 93 مصابا بالحمي كان هناك عدد 83 (89.2%) مصل ايجابي للاجسام المضادة IgM لفيروس حمي الضنك بينما 10(10.8%) كان سالبا.بالنسبة لأيجابية حمي الضنك حسب الفئة العمرية، كان هناك (43.3%) 36/44 في الفئة العمرية1-5 سنوات، (38.6%) 32/34 في الفئة العمرية 6-12 عاما، و(16.1%) الفئة العمرية 12-17 عاما كانت موجبة للاجسام المضادة لفيروس حمي الضنك. لا يوجد ارتباط بين الفئات العمرية و الاجسام المضادة لحمي الضنك ،القيمة الاحتمالية (P=0.075) .بالنسبة للجنس، (49.4%) 42/47 للزكور و (50.6%) 41/46من الاناث موجبة للاجسام المضادة لفيروس حمي الضنك. ولكن هناك ارتباط لا معني له بين الجنس و الاجسام المضادة لحمي الضنك،القيمة الاحتمالية (P=0.97). وبالنسبة لمدة الاعراض كان هناك (89.2%) 74 في المجموعة 3_7 ايام و(9.8%) 9 في مجموعة اكثر من 7 ايام، لا يوجد ارتباط بين مدة الاعراض والاجسام المضادة لفيروس حمي الضنك،القيمة الاحتمالية(P=0.65). وبخصوص الاقامة كان هناك (26.5%) 22 في مدينة بورتسودان و(74.5%) 61 ف مدينة كسلا، لا يوجد ارتباط بين منطقة الاقامة والاجسام المضادة لفيروس حمي الضنك ،القيمة الاحتمالية (P=0.23).

خلصت هذة الدراسة أن معدل أنتشار الأجسام المضادة لـحمى الضنك بين المرضى المصابون بالحمى مرتفع بشكل ملحوظ لانه في تلك الفترة كانت ولاية البحر الاحمر وولاية كسلا تعانيان من وباء الحمي.

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LIST OF ABREVIATIONS

Abbreviation	Full name
CS	Cyclization sequences
DC	Dendritic cell
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NSI	Non-structural protein antigen
RNA	Ribonucleic acid
SEA	Southeast Asia
TDR	Special program for Research and Training in Tropical Diseases
TGN	Trans Golgi network
UAR	Upstream AUG Regions
UNEP	United Nation Environment Program
UNICEF	United Nation Children Fund
WHO	World Health Organization
WP	Western pacific

CHAPTER ONE
INTRODUCTION

CHAPTER I

INTRODUCTION

1.1. Introduction

Dengue virus is the most rapidly spreading and frequently encountered mosquito-borne viral infection in the world. The incidence of dengue viral infection has increased 30 fold over the last decade with increasing geographical expansion to other countries with explosive outbreaks (WHO, 2016a). Before 1970, severe dengue epidemics had been reported from only 9 countries but currently the virus is endemic in more than 100 countries in the WHO regions of Africa, the East Mediterranean, South East Asia and Western Pacific (WHO, 2017). Almost 2.5 billion people live in endemic areas resulting annually in an estimated 50 to 100 million cases of dengue fever (DF), 250, 000 to 500,000 cases of dengue haemorrhagic fever (DHF) causing 20,000 deaths and 264 disability adjusted life years per million population per year are lost (Wichmann *et al*., 2003). Has been reported from over 60 countries in Africa, Asia, Europe and the Americas. After its detection in 1953 from Africa , the virus has been circulating at a relatively low levels in Africa till 2000 where large outbreaks occurred in Democratic Republic of Congo, Gabon and Kenya (WHO, 2016b).

Dengue viruses have become major international public health concern due to their epidemics and introduction of the viruses in new areas. Due to its associated increase in the incidence, distribution and clinical severity of the disease linked with these viruses, it's on the priority lists of the World Health Organization (WHO), United Nation Children Fund (UNICEF), United Nation Environment Program (UNEP), World Bank and WHO special program for Research and Training in Tropical Disease (TDR) (Restrepo*et al*., 2014).

Re-emergence of these arboviruses exert enormous burden on populations, health systems and economies in most tropical countries. The spread of virus to the Americas, Africa, East Mediterranean regions and Asia represent a global pandemic threat (WHO , 2011). Reemergence of the virus in areas where the incidence had been controlled or eradicated is largely due to vector management associated with reduced allocation of funds towards vector and increased resistance in mosquitoes (Charette *et al*, 2017). Dengue viruses are transmitted by *Aedes aegypti* and *Aedes albopictus* which have adapted to peri-domestic setting making transmission more pronounced in the tropical urban centers with high population. More than 50 % of the world's population lives in areas infested with these mosquitoes. The presence

of these vectors together in high population density represent a high probability of an outbreak and spread of these viruses (Musso *et al*., 2015).

Transmission of dengue virus have been reported to be endemic in 34 countries in all African regions (Amarasinghe, *et al*., 2011). These viral infections have also been diagnosed in travelers from Europe and North America returning from several central and West Africa countries including Sudan (Baronti *et al*., 2017). Re-emergence of these viruses now poses greater risk due to the increase in number of cases and its associated severity and complications. Though the disease burden has increased, dengue epidemiology in African region have not well been documented. There is paucity of information, public health interventions and health system preparedness. Many febrile cases are presumptively diagnosed and treated for malaria due to lack of investigation tools for these virusesin hospital facilities. Enormous attention given to malaria and malaria burden in Africa contributes to a minor concern for dengue cases (D'Acremont, *et al*., 2010).

1.2 Rationale

Dengue cases share symptoms as malaria and can easily be misdiagnosed as malaria. Misdiagnosis hinders epidemiological importance of dengue disease and greatly affect the clinical picture of, and outcome for infected patients. Again misdiagnosis risk delaying of specific supportive treatment and in the case of dengue can lead to ten-fold impact on the likelihood to the progression of dengue fever to dengue haemorrhagic fever which can lead to death. This therefore place much importance on the investigation of non-malaria cause of fever such as dengue which are not part of the routine hospital laboratory investigation in Sudan. (Baronti *et al*., 2017) This will improve awareness of these agents, case detection and management, promote surveillance and prevent inappropriate prescription of non-steroidal anti-inflammatory drugs which could lead to severe bleeding in patients with severe thrombocytopenia.Many febrile cases are presumptively diagnosed and treated for malaria due to lack of investigation tools for these virus in hospital facilities. Enormous attention given to malaria and malaria burden in Africa contributes to a minor concern for dengue cases (D'Acremont *et al*., 2010). Severe form of the illnesses caused by dengue virus (DENV) especially the latter is often characterized by haemorrhagic manifestations. Data from this study to some extent may supports the role played by dengue virus in the occurrence of febrile illnesses in Sudan and highlight the circulation of these arthropod borne infections other than malaria.

1.3 Objectives

1.3.1 General Objective

To detect the serofrequency of Dengue viral infectionamong febrilepatients in Eastern Sudan.

1.3.2 Specific Objectives

1. To detect anti dengue virus IgM antibodiesbyEnzyme Immunosorbent assay (ELISA)

2. To determine the frequency of anti -Dengue virusIgM antibodies among febrile patients.

3. To associate between dengue virus infections and possible risk factors including Sex, age, residence, the symptoms of disease and the duration of symptoms.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER II
LITERATURE REVIEW

2.1 Dengue virus

Dengue is an acute febrile disease caused by the mosquito-borne dengue viruses (DENVs), consisting of four serotypes (DENV 1 to 4); dengue virus is a member of the family Flaviviridae and of the genus Flavivirus. It is a spherical virus of about 50 nanometers in diameter consisting of three structural proteins; the capsid (C), the pre membrane/membrane (prM/M)andan envelope (E).The 10.7 kilo base positive sense single stranded ribonucleic acid (RNA) genome also encodes for 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5). The order of gene is in 5-CprM (M)-E-NS1-NS2A-NS2b-NS3-NS4A-NS4B-NS5-3 (Diamond and Pierson, 2015). Upon infection with the virus, it activates the interferon signaling pathway but the virus develops resistance by NS2A,NS4A and NS4B that blocks the interferon cascade to escape the immune response (Idrees and Ashfaq, 2012).

There are 4 known serotypes of the Dengue virus circulating in the tropical and subtropical region in the world. These serotypes (Dengue virus 1-4) differ from one another by 25-40% amino acid level (Bhatt *et al*., 2013). The co-circulation of diverse serotypes is common in hyperendemic regions and mostly an outbreak arises from a dominant serotype in a 2-4 year cycle (Moi *et al*, 2016).

2.1.1Antigenic structure and serotypes of Dengue virus

The serotypes are further separated into subtypes that differ by 3%. There are 3 subtypes of Dengue virus 1, 6 for Dengue virus 2 (one of which is found in non-human primates), 4 for Dengue virus 3 and 4 for the serotype 4. Phylogenetic studies have shown that subtypes normally circulate within a defined area (Lambrechts *et al*., 2012).

The four DENV serotypes can cause a wide range of diseases in humans even though DENV infections may also be asymptomatic. The diseases range in severity from undifferentiated acute febrile illness, classical dengue fever (DF), to the life-threatening conditions DHF/DSS (Gubler *et al*., 2012). Dengue illness was previously categorized on a I–IV grade scale, but a simplified categorization for dengue case classification has been proposed by WHO's Special Program for Research and Training in Tropical Diseases (TDR) in 2009 where DHF and DSS cases are grouped together as ‘severe dengue’ (group C) to avoid false-negative DHF/DSS diagnosis (WHO, 2017).

After an incubation period of 3–15 days (usually 5 to 8), classical DF begins with an abrupt onset of high fever. During the febrile phase, dehydration may cause neurological disturbances and febrile seizures in young children (Guzman and kouri 2012). The condition is self-limiting through debilitating illness with headache, retro-orbital pain, myalgia, arthralgia, petechiae rash, and leucopenia. A macular-papular recovery rash appears 3–5 days after the onset of fever, and it usually starts on the trunk before spreading peripherally (Gubler, 2012). DF is sometimes referred to as 'break bone fever' due to its incapacitating symptoms with severe muscle and joint pain (Moi *et al*, 2016).

Early symptoms of DF and DHF are indistinguishable, but DHF is associated with hemorrhagic manifestations, plasma leakage resulting from an increased vascular permeability, and thrombocytopenia (<100,000 platelets/mm3). Thrombocytopenia is not necessarily restricted to severe dengue, and minor bleeding may occur in mild infections, which can be severe in those with peptic ulcer disease (Moi *et al*, 2016).

DSS is distinguished from DHF by the presence of cardiovascular compromise, which occurs when plasma leakage into the interstitial spaces results in shock. DSS is a fatal condition with mortality rates as high as 20% but can also be less than 1% in places with sufficient resources and clinical experience. Common clinical warning signs for DSS include a rapidly rising haematocrit, intense abdominal pain, persistent vomiting, and narrowed or absent blood pressure (Gubler, 2012).

2.1.2 Replication cycle

DENV is an envelope, single-stranded positive-sense RNA virus. The RNA genome consists of approximately 10,700 nucleotides and encodes a 3,411 amino acids long precursor polyprotein containing three structural proteins capsid (C) precursor membrane (prM) and envelope (E) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Diamond and Pierson, 2015).

The structural proteins are components of the mature virus particle whereas the NS proteins are expressed only in the infected cell and are not packaged to detectable levels into mature particles. The structural proteins are not involved in replication of the viral genome (Pang*et a*l., 2011).

The open reading frame is flanked by two untranslated regions (5' and 3' UTR) of approximately 95–135 and 114–650 nucleotides, respectively. The 5'-end contains a type I cap, similar to cellular mRNA, and the viral RNA (vRNA) is translated by a cap-dependent initiation scanning

the 5'-UTR. The 3'-end lacks a poly(A) tail but ends in a conserved stem-loop (SL) structure. Both the 5'- and 3'-UTRs are required for efficient translation and replication. The UTRs have characteristic secondary structures that confer distinct functions and show high sequence conservation among different DENV serotypes. The 5'-UTR contains a large stem-loop (SLA) that is proposed to act as the promoter for the viral RNA-dependent RNA polymerase (RdRp) NS5. Both the 5'- and the 3'-UTRs contain complementary Upstream AUG Regions (UAR) and cyclization sequences (CS) that hybridize in order to mediate genome cyclization and RNA synthesis (Gamarnik, 2010).

The various steps in the flavivirus life cycle include virions binding to cell-surface attachment molecules and receptors, and are internalized through endocytosis due to the low pH of the endosome, viral glycoproteins mediate fusion of viral and cellular membranes, allowing disassembly of the virion and release of vRNA into the cytoplasm the vRNA is translated into a polyprotein that is processed by viral and cellular proteases, and the viral NS proteins replicate the genome RNA. Virus assembly occurs at the endoplasmic reticulum (ER) membrane, where C protein and vRNA are enveloped by the ER membrane and glycoproteins to form immature virus particles. Immature virus particles are transported through the secretory pathway, and in the acidic environment of the trans-Golgi network (TGN), furin-mediated cleavage of prM drives S maturation of the virus. Mature virus is released from the cell. (Gamarnik, 2010).

2.1.3 Epidemiology and burden of Dengue virus

Transmission of dengue virus, *Aedes aegypti* and *Aedes albopictus* are the main vectors of dengue. *Aedes aegypti* isa dark mosquito with white and black striped legs and a silvery lyre shaped pattern of scales on the dorsal side of the thorax. It is common and found to infect almost all countries in Sub Sahara Africa. It is present in a wide range of environment preferably poor sanitation and densely crowded areas, the adults are mostly within or near human habitat and bite during day time (Kamgang *et al*., 2010). *Aedes albopictus* is common in South East Asia and Americas preferably in the suburban and rural settings. Like the Aedes. aegypti, they have white dorsal stripes but not lyre shaped pattern. Though it has a high affinity for biting humans, it also feed on dogs, cats, squirrels and deer. Its peak biting time is early morning and late afternoon (CDC, 2012).

Before 1970, 9 countries had reported cases of severe dengue, the disease has spread to more than 100 countries in Asia, Africa, Europe and the pacific after 2014 (WHO, 2017). In

2010, it was estimated that about 2.5 billion people live in an endemic countries resulting annually in an estimated 50 to 100 million Dengue fever infections (Wichmann *et al*., 2013). There were approximately 390 million dengue infections worldwide 2010, of which 96 million manifest clinically. Of the apparent infections, 70% occurred in Asia and 14% in Africa and America each (Brady*etal.*,2012). Dengue infections are underreported in Africa due to inadequate surveillance and difficulty in the differential diagnoses as against other endemic infections in the continent (Bhatt *et al*., 2013).

DENV is presently the most common cause of arboviral disease globally, and all four serotypes of DENV can be found worldwide. More than 100 countries are endemic, primarily affecting 2.5 billion inhabitants in the tropical and subtropical regions as well as 120 million travelers to these regions every year (Guzman *et al*., 2012). The World Health Organization (WHO) estimates an annual incidence of approximately 100 million infections, with approximately 500,000 people with dengue hemorrhagic fever (DHF) requiring hospitalization, a large proportion being children. DHF may develop into dengue shock syndrome (DSS) whereof the mortality rate is approximately 1–2.5%. Successful treatment of patients with DHF and DSS is labor intensive and expensive, but without proper treatment, fatality rates may exceed 20% (WHO, 2017).

The average number of severe cases of dengue reported to WHO continue to increase exponentially from an average of 1,000 cases in 1950 to more than 3 million cases per year globally in 2013. From 2000 to 2008, 1,656,870 Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) cases were reported to WHO compared to 479,848 cases in 1990 to 1999. A record of 69 countries from the WHO regions of South-East Asia (SEA), Western Pacific (WP) and the Americas in 2008 reported cases of Dengue fever (WHO, 2011).

It is estimated that 9,221 people died of dengue infections per year between 1990 and 2013. Mortality rate is higher in children less than 1 year and in patients more than 45 years. A total of 576,900 years of life lost to premature mortality and 56,600 years lived with disability is credited to dengue in 2013 (Stanaway *et al*., 2016). In 2015, WHO regions of SEA, WP and Americas reported 3.2 million dengue activity with 2.5 million of these cases from the Americas alone of which 10,200 were diagnosed as severe resulting in 1181 deaths (WHO, 2017).

The number of reported dengue cases has increased dramatically since the 1980s due to several complex reasons (Thomas *et al*.,2013). The contribution of climatic change is controversial, and

it is not known to what extent this enhances the spread of mosquitoes, and indirectly the DENVs (Thomas *et al*., 2013).

Dengue is hyperendemic in South East Asia. In 2013, 8 countries in the region had reported cases of Dengue by 2009 the infection has spread to all member countries except Democratic People of Korea. Timor-Leste, Bhutan , Nepal, Indonesia, all in SEA reported outbreaks in 2004 (WHO, 2011). Indonesia in 2004 reported 58,301 cases of Df/DHf resulting in 658 deaths thus case fatality rate of 1.1% (WHO, 2014). In Asia, trends indicate surge in dengue cases in China, the Cook Island, Fiji, Malaysia and Vanatu. In 2015, Malaysia reported 111,000 suspected dengue cases, 16% higher than the number of cases reported in 2014. India in 2015 also recorded its worst outbreak of Dengue since 2006 (WHO, 2017).

Dengue also continues to affect several American countries despite concerted dengue control efforts. Epidemics have occurred in Mexico, Honduras, Costa Rica, Brazil, Ecuador and Venezuela. Of the 14.2 million cases of dengue with 7000 deaths reported in this region between 2000 and 2014, 70% were from Brazil, Columbia and Mexico (PAHO, 2014).

In Europe reports of dengue have been cases imported by travelers or expatriates returning from endemic countries. According to European Network on Imported Infectious Diseases Surveillance (TropNetEurop), the number of cases increased from 64 in 1999 to 224 in 2002. For 2008, there was a slight decline to 116 cases (Jelinek, 2019). Among travel-acquired Dengue virus cases reported from Europe , 45% was acquired by patients who travelled to countries in South East Asia , 19 % from South and Central America , 16% from India , 12% from the Caribbean and 8% from Africa (Wichmann *et al.,* 2003). Ghana and other 26 African countries have been reported as locations where expatriates and travelers from countries which Dengue was not endemic has acquired the infection (Amarasinghe*et al.,* 2011).

In Europe, dengue has been ranked second after Malaria for frequent admission into hospital after returning from abroad (WHO, 2014).

Imported viral cases coupled to competent vectors and immunological naïve individuals have led to the report of autochthonous cases in Europe. France and Croatia reported 2 and 17 autochthonous cases in 2010 respectively. The outbreak of Dengue in Portugal in 2012 led to the report of 2,200 confirmed laboratory cases (Schaffner and Mathis 2014).

In Africa, after the virus was first detected in 1960 in Nigeria, it caused outbreak in more than 20 countries (Amarasinghe *et al.,* 2011). Thousand and sixty six (1066) probable cases and

case fatality rate of 1.2% has been reported from the recent outbreak in Burkina Faso (WHO, 2016). Literature suggests that the virus is endemic in 34 countries in all regions of Africa. Twenty two (22) out of the 34 have reported confirmed laboratory cases (Amarasinghe *et al*., 2011). In Ghana a seroprevalence of 3.2 % and 21.6% to IgM and IgG respectively to the virus were detected in 2015 in malaria parasite positive children in urban centers in Ghana which suggest exposure and transmission of the virus (Stoler *et al*., 2015).

2.1.4 Immunity to dengue virus

In the secondary infection by heterogenic serotype, the antibodies directed against the previous infection do not confer immunity against the current infection. These antibodies from the previous infection enhances severe form of the infection by forming immune complex with the virus that binds to the Dengue target cells in a phenomenon called antibody dependent enhancement. Antibody dependent enhancement is mostly experienced by infants born to dengue-immune mothers and adults with waning homotypic antibodies (Moi *et al*., 2016).

Primary and Secondary infections are differentiated by IgM/IgG ratio. Where the ratio is greater than 1.2, the infection is defined as primary and the vice versa for the secondary infection (WHO, 2016a).

A few days after the onset of the fever, specific IgM antibody to the virus appears as the initial response to the primary infection. It is detected as early as 3-5 days after the onset of the fever, mostly suppressing the viraemia and may last for 30 -90 days. Since IgM may persist in the serum for more than 30 days, a positive result on a single serum sample is only provisional and does not necessarily mean the infection is current and ongoing. The most reliable way to confirm an active infection is by a significant four fold or greater rise in the antibody in the paired sera. IgG appears 2 weeks after the infection, reaching its peak at the 3rd week. The IgG antibody is type specific and does not give protection against reinfection by a different serotype. The IgG and IgM antibodies give immune protection by blocking cellular attachment, viral fusion or by antibody dependent cellular cytotoxicity (WHO, 2006).

2.1.5 Infection cycle of dengue virus

Transmission of the virus occurs when an *Aedes aegypti* mosquito feeds on infected human during the viraemic phase of the illness that manifest 2 days before the onset of the fever and lasts for 4 to 5 days after the onset of the fever. The minimum virus concentration needed to establish infection in the mosquito has not been formally established (Runtuwene *et al*., 2014).

After the ingestion of the virus contaminated blood, the virus binds to receptors on the cellular surface of the midgut epithelium. The virus replicates within the midgut and are shed through the hemocoel to affect other secondary tissues such as the salivary glands. When the salivary glands become sufficiently infected, upon the next blood meal, the virus may be transmitted to a new host through the saliva of the infected host (Carrington *et al.*, 2014).

Human Dengue viral infection in the absence of the vector has been reported by literature. Cases of the transmission through needle stick injury (Chen and Wilson, 2004), bone marrow and solid organ transplant (Sabino *et al*., 2016). In perinatal transmission, the virus has been detected in placenta and cord blood of infected infants. The virus can also be shed through breast milk of infected mothers (Ribeiro *et al*., 2013).

2.1. 6 Pathogenesis of dengue virus

During the feeding of mosquitoes on humans, DENV is presumably injected into the bloodstream, with spillover in the epidermis and dermis, resulting in infection of immature Langerhans cells and epidermal dendritic cells. Infected cells then migrated from site of infection to lymph nodes, where monocytes and macrophages are recruited, which become targets ofinfection. As a result of this primary viremia, several cells of the mononuclear lineage, including blood-derived monocytes, myeloid DC and splenic and liver macrophages are infected.Secondary infection with heterologous DENV,high concentration of immunoglobulinG (IgG),following infection mononuclear cells, predominantly died by apoptosis, DC,were stimulated to produce the bulk of mediators and hemostatic, responses of the host,determined the ratio of different proinflammatory and inflammatory cytokines, chemokines,and other mediators (Byron *et al*, 2009).

2.1. 7 Clinical manifestations

After an incubation period of 4 to 10 days, infection by any of the 4 serotypes can cause a wide range of illnesses. The infection can be unapparent or may cause undifferentiated febrile illness, dengue fever,dengue haemorrhagic fever and dengue Shock syndrome. The severity of the disease depends on the age of the patient, race and secondary infection by a different serotype (Simmons *et al*., 2015).

2.1.7.1 Dengue Fever

Following the incubation of the virus, the infection goes through three phases; the febrile, the critical and the recovery phase. The early phase of the infection characterized by fever lasts for 4 to 10 days and it is followed by non-specific signs and symptoms such as headache, retro-ocular pain, malaise, myalgia, arthralgia, anorexia and vomiting. The early symptom of the infection is variable and makes it difficult to differentiate it from other febrile illnesses. At the critical phase of the infection the body is flushed with a visible rash that may be maculopapular. Towards the end of the febrile phase, the rash is replaced by petechiae which may appear on the feet, and on the hands of the patient. Occasionally there is an unusual haemorrhagic manifestation such as gastrointestinal bleeding, hypermenorrhea, and massive epistaxis. Dengue Fever is common in adults, adolescent and children. The disease is self-limiting, only small proportion progress to the severe form (Hadinegoro, 2012)

2.1.7.1.1 Complications of Dengue Fever

2.1.7.1.1.1 Severe Dengue (Dengue Haemorrhagic Fever)

Dengue Haemorrhagic Fever (DHF) is characterized by high fever, haemorrhage, defects in homeostasis, hepatomegaly and evidence of vascular leakage such as ascites, pleural effusion and hypo-albuminaemia which can lead to shock. WHO has graded the severity of the DengueHemorrhagic Fever to the following: Grade I and II are differentiated by presence of thrombocytopenia and concurrent haemo-concentration. Circulatory failure and profound shock with undetectable blood pressure and shock differentiate grade III from grade IV. Grading of the severity of the disease has been found clinically and epidemiological important in epidemics in SEA, WA and America regions of WHO(WHO, 2011).

Dengue heamorrhagic fever is common in infants that acquired maternal antibodies and have been subsequently been affected by the virus. In adults, it is caused by secondary infection by a different serotype of the virus. Primary infection by Dengue virus 1 and virus 3 have also been documented to cause DHF (Sanyal*et al.*, 1991). Besides secondary infection, congenital heart disease, heamolytic disease, chronic disease such as bronchial asthma and diabetes has been implicated as risk for developing DHF. The risk is also higher in whites than in blacks. The Dengue virus 2 is known to replicate in a greater concentration in peripheral blood of whites than in blacks (Kouri*et al.*, 1987). In DHF, there is plasma leakage which can lead to

hypovolemic shock if not quickly managed. Patients in shock are at greater risk of dying if no prompt treatment and management is given (Sanyal *et al*., 1991).

2.1.7.1.1.2 Dengue shock syndrome

Dengue shock syndrome is defined as DHF accompanied by an unstable pulse, narrow pulse pressure (<20 mmHg), restlessness, cold, clammy skin, and circumoral cyanosis. Progressively worsening shock, multiorgan damage, and disseminated intravascular coagulation account for a high mortality rate associated with DSS. The shock persists for a short span of time and the patient promptly recovers with supportive therapy (Rajapaksa, 2011).Shock syndrome is a dangerous complication of dengue infection and is associated with high mortality. Severe dengue occurs as a result of secondary infection with a different virus serotype. Increased vascular permeability, together with myocardial dysfunction and dehydration, contribute to the development of shock, with resultant multiorgan failure,the onset of shock in dengue can be dramatic, and its progression relentless (Rajapaksa, 2011).The pathogenesis of shock in dengue is complex. It is known that endothelial dysfunction induced by cytokines and chemical mediators occurs. Diagnosis is largely clinical and is supported by serology and identification of viral material in blood. No specific methods are available to predict outcome and progression. Careful fluid management and supportive therapy is the mainstay of management. Corticosteroids and intravenous immunoglobulins are of no proven benefit. No specific therapy has been shown to be effective in improving survival. (Rajapaksa, 2011).

2.1.8 Laboratory diagnosis of dengue virus infection

Diagnosis of the infection at the acute stage is made by tourniquet testing, testing for the non-structural protein antigen (NS1) and complete blood count. A positive tourniquet test, increase in hematocrit, thrombocytopenia and leucopenia with neutropenia with lymphocytosis is suggestive of dengue infection. Positive NS1 antigen confirms dengue virus as the cause of the infection. (Hadinegoro, 2012).

2.1.8.1 Diagnostic method for detection of Dengue infection

Rapid and accurate diagnosis of dengue infection is key to clinical management for appropriate clinical care, epidemiological survey for determining the virus during an outbreak for prompt public health intervention and for research and vaccine trials. Laboratory diagnosis is also important for differential diagnosis of dengue from other febrile illnesses such as leptospirosis, meningococcemia, influenza and sepsis. Laboratory diagnosis is central to

clinicians to identify patients who should be closely monitored for signs of severe dengue which can lead to shock and death. Laboratory diagnosis method for detection and confirmation of the virus include virus isolation by culture, viral nucleic acid detection and serological methods. The choice of the method is dependent upon the reason for testing (clinical diagnosis, survey or vaccine development), the phase or stage of the infection (acute or convalescent) and the technical expertise available (Plennevaux *et al*., 2016).

2.1.8.1.1 Isolation of Dengue virus

Virus isolation is the most reliable method for detecting and confirming infection. It provides direct and definitive evidence of virus circulation and critical for viral characterization and determination of epidemiological characterization (Phanthanawiboon *et al*., 2014).

Cell culture is the commonly used method for dengue virus isolation. C6/36 and Vero Cells are the most widely used cell lines for isolating dengue virus though the virus can replicate in other vertebrate and invertebrate cell lines. C6/36 cell line is derived from the *Aedes albopicutus* and the Vero cells from the African green monkey kidney epithelia cells. Cell culture is most appropriate when samples are taken at the early phase of the disease and processed without delay (Phanthanawiboon *et al*., 2014) though it has the advantage of detecting virus which may occur in low concentrations in viraemic sera, it is time consuming; require substantial skill and competency and infrastructure with Biosafety level 2 or 3. Specimen appropriate for virus isolation include acute phase serum, plasma or washed buffy coat from the patient, autopsy tissue from fatal cases (liver, spleen, thymus and lymph nodes) and mosquitoes collected from affected areas.A marked cytopathic effect is seen in the cell lines when samples are positive for any of the serotype for the Dengue virus (WHO, 2011).

2.1.8.1.2 Viral nucleic acid detection

Molecular diagnosis has contributed significantly to Dengue fever investigation and clinical treatment due to its sensitivity, specificity and reliable system for the detection and characterization of the virus with a much more rapid turnaround time. Compared to the virus isolation, the sensitivity of the molecular methods varies from 80% to 100% depending on the region of the genome targeted by primers, the procedure used to amplify and detect the products, and the methods for subtyping (Shuand Huang 2004).

A number of reverse transcriptase – polymerase chain reaction (RT- PCR) assays have been reported for detecting the dengue virus (Shuand Huang 2004) , among these the two step

nested RT-PCR reported by (Lanciotti*et al.,* 1992) and later improved to a single step multiplex RT-PCR for detection and typing of the virus by (Harris *et al*., 1998), is well known. This assay uses the Dengue virus core to pre-membrane gene regions as the target sequence for detecting the 4 serotypes of the Dengue virus by analyzing the unique sizes of the amplicons in an agarose gel. Currently the automated real time reverse transcriptase polymerase chain reaction (rRT-PCR) has replaced the conventional (RT-PCR) methods due to its ability to provide quantitative measurements, a lower contamination rate, high sensitivity and specificity and easy standardization. The rRT has steadily replaced the conventional RT-PCR as the new gold standard for the rapid diagnosis of Dengue virus infection with acute phase serum samples The rRT –PCR uses primer pairs, fluorescent probes and 5‘ nuclease to detect a single nucleotide a time (singleplex) or a multiple or all the 4 at time in a specialized PCR machine without electrophoresis (Shuand Huang 2004).

2.1.8.1.3 Viral antigen detection

DENV NS1 is a 48-kDa glycoprotein that is highly conserved among all flaviviruses. NS1 is essential for viral replication with an unknown mechanism that possibly involves interactions with NS4A and NS4B. The NS1 protein, a product of the NS1 gene is a glycoprotein of about 50 kDa. The protein is secreted by only mammalian cells upon infection with the virus. The NS1 protein when produced do not form part of the virus assembly but is released from the Dengue virus infected cells. The antigen appears as early as first day after the onset of the febrile illness and decline to undetectable levels by 5- 6 days (Wang and Sekaran, 2010). It has been demonstrated that the antigen can be detected in the acute phase serum o patients with either primaryor secondary Dengue infections (Oyero andAyukekbong,2014).

Lateral flow immune-chromatographic assay is available for the detection of NS1 antigen however it is disadvantaged such that it cannot differentiate between the different serotypes of the virus (WHO, 2009). However, the sensitivity for the lateral flow immune-chromatographic test range from 81% to 94% and makes it a feasible method for early diagnosis of Dengue fever (Ferraz *et al*., 2013).

2.1.8.1.4 Serological tests

Different serological methods are used to detect and describe the dengue virus specific antibodies. These methods include the Hemagglutination test, indirect immunofluorescent antibody test, complement fixation, Western blotting and the enzyme linked

immunosorbent assay (ELISA). Among the methods, the most widely used for routine diagnosis of dengue infections is the capture IgM and the indirect IgG ELISA. These methods are now common due to their high sensitivity, simplicity, specificity and feasibility for automation. The capture IgM ELISA and the indirect IgG ELISA are key methods used for surveillance (Muianga *et al*., 2016).

Direct immunofluorescent test was essential requirement for detection of dengue virus, this method with high sensitivity, specificity and respectively during febrile stage of disease. (Chunhakan *et al*., 2009)

Detection of dengue virus IgM and IgG is an important laboratory tool for identifying patients with acute dengue virus infection because of the relatively short time window wherein dengue virus IgM is measurable. Denguevirus IgM reaches detectable levels in nearly all dengue viruses -infected patients within 5 days of symptom onset and reaches peak levels approximately 2 weeks later. Peak IgM levels are usually lower in secondary infections than in primary infections. it is generally agreed that dengue virus IgM wanes to undetectable levels within months of disease onset, published estimates of dengue virus IgM persistence range from 2 months to 6 months. (Muianga *et al*., 2016)

In areas where the virus is not endemic, these methods are used for clinical surveillance for viral illness where positive results are treated as recent infection (Muianga *et al*., 2016)

2.1.9 Treatment and control of Dengue virus

There is no specific antiviral agent for the treatment of dengue illnesses. Clinical management is by supportive treatment where intravascular volume is replaced (WHO, 2015). Supportive treatment has reduced the case fatality rate of dengue from 20% to less than 1% prevention of dengue illnesses approach targeted against the vector (Simmons *et al*., 2015).

Wolbachia pipientis is a bacterial endosymbiont common to many insect species. Wolbachia transinfections in mosquito disease vectors have great value for disease control given the bacterium ability to spread in to wild mosquito populations and to interfere with infections of pathogens, such as dengue virus (Silva *et al*., 2017).

2.1.9.1 Vaccine

As of 2021, one version is commercially available, known as CYD-TDV, and sold under the brand name Dengvaxia. Currently the CYD-TDV is the only approved vaccine for use against the prevention of Dengue infection by the 4 different serotypes of the Dengue virus. This vaccine

has been evaluated in phase III randomized trials conducted in Asian countries and Latin America.The results for vaccine efficacy against symptomatic confirmed dengue in Asia and Latin America was 56.5% and 60.8% respectively (WHO, 2015).

The vaccine under the trade name DENGVAXIA is a prophylactic, tetravalent, live attenuated chimeric Dengue vaccine in a Yellow fever 17D backbone developed by Sanofi Pasteur, the vaccine division of the pharmaceutical company Sanofi. The live attenuation of the CYD-TDV is obtained by replacing the genes that encode the prM and E proteins of the attenuated Yellow fever 17D virus genome with the corresponding genes of the 4 different serotype of the Dengue virus. The vaccine contains no adjuvant or preservative and can last for 36 months when stored between 20C and 80C and secured from light (WHO, 2016b).

Theefficiency of the vaccine is approved for use in endemic areas in individuals between the age of 9 and 60 years. The vaccine has been reported not advisable for use in individuals who are allergic to any of the component of the Dengue virus, immunocompromised, pregnant and lactating mothers (WHO, 2016).

2.1.10 Prevention and control

WHO recommends an integrated vector control program which encompass biological, chemical, source reduction and larviciding, environmental management and manipulation to reduce or interrupt the virus transmission by the *Aedes* species (WHO, 2011).

2.2 Previous Studies

In a study done by Adam *et al* (2018) to determine the seroprevalence of dengue in Eastern Sudan and the breadth of neutralizing antibody responses, Adam study that results 89% in Port Sudan and 61% in Kassala. The majority of the sera broadly neutralized all four dengue virus serotypes indicating multiple infections. They concluded, the majority of the population in eastern Sudan has been infected with dengue viruses, many people repeatedly.

Another study was low seroprevalence rate (8.9%) done by Al-Sheikh and Omer (2020) to detect dengue virus IgM (DENV-IgM) antibodies in febrile hospitalized patients in Khartoum State (Sudan), a descriptive, cross-sectional study investigating 90 febrile patients attending some Khartoum State hospitals. From the 90 febrile patients investigated, 53(58.9%) were males and 37(41.1%) were females.8 febrile patients (8.9%) were found positive for dengue virus IgM antibodies.

A cross sectional study was conducted by Eldigail et *al* (2018) in El-Gadarif State. And localities were determined as potential risk factors for contracting DENV infection. Their conclusions was, the prevalence rate of DENV antibodies among residents of El-Gadarif State is significantly high (47.6%).

Another cross sectional study was conducted in Kassala State, Sudan by Eldigail et *al* (2020). The prevalence of recent DENV infection was estimated to be (11.42%). Potential risk factors to DENV seropositivity include, age (OR = 3.24, CI = 1.81–5.77, p-value = 0.001). Their Conclusion was, the study showed a high rate of circulating DENV IgM antibodies among the participants of the study (11.42%), suggesting recent transmission of DENV in Kassala State, Eastern Sudan.

In a retrospective study of Santiago *et al* (2013) with 102 dengue cases confirmed by IgM anti-DENV seroconversion in the convalescent sample, the RT-PCR Assay detected DENV RNA in 98.04% of the paired acute samples. Using sequencing as a positive indicator, the RT-PCR Assay had a 97.92% positive agreement in 86 suspected dengue patients with a single acute serum sample.

Another study was received from hospitals in Nairobi, northern and coastal Kenya. 40% of the samples tested positive for dengue by either IgM ELISA (14.6 %) or by RT-PCR (25.1 %). (Konongoi*et al*., 2016)

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Study design

This study was descriptive cross section study conducted at Eastern Sudan**.**

3.2 Study area and duration

Study was conducted in Eastern Sudan **(**Port Sudan Teaching Hospital and Kassala Teaching Hospital) duringDecember 2019 to April 2022.

3.3 Study Population

Patients suffering from fever and attending Port Sudan teaching hospital and Kassala Teaching Hospital.

3.3.1Inclusion criteria

Anyfebrile individualall age group, different sex and agreed to participate were included in this study

3.3.2 Exclusion criteria

Any patients suffering from Tuberculosis, rheumatoid arthritis was excluded

3.4 Ethical consideration

Ethical approval was taken from ethical comity of College of Medical Laboratory Science Sudan University of Science and Technology.

3.5 Sample size

Ninety three (n = 93) febrile patients from Port Sudan and Kassala state were enrolled in the study.

3.6 Data collection

A non-self-interviewing Questionnaire was used to collect demographic, clinical data.

3.7 Sampling Technique

This study based on non-probabilityconvenience sampling technique.

3.8 Laboratory processing

3.8.1 Specimen collection

Three ml of venous blood was collected from patients in a plain container, and allowed to clot at room temperature. Then the samples were centrifuged and serum was separated in a sterile container and stored at -20 C until analysis.

3.8.2. Detection of dengue virus IgM by Enzyme linked Immunosorbent Assay

The (EUROIMMUN ELISA Germany), kit was used to detect dengue virus IgM the ELISA test kit provides semi quantitative in vitro determination of human antibodies of the immunoglobulin class IgM against dengue virus in serum or plasma to support the diagnosis of a dengue virus infection.

3.8.2.1. Procedure of ELISA

A 100µl of the calibrators, positive control, negative controls and diluted patients' samples were transferred to microplate wells and incubated for 30 min at 32^{o}C, a 300µl of working strength wash buffer was used to Wash microplate wells. And this process repeated three times, a 100µl of enzyme conjugate was pipetted into each of the microplate wells and incubated for 30 min at room temperature. The microplate wells were washed again as previously described in step two, a 100µl of chromogen / substrate was pipetted into each microplate wells and incubated for 30 min at 32^{o}C, A 100µl of stop solution was pipetted into each microplate wells and incubated for 5 min, Results was recorded at 450 nm wavelength filter and also at 650 nm for reference reading by ELISA reader, Calibrator 2 was measured to evaluate the results according to following formula:

$$\textbf{Ratio} = \frac{\textbf{Extinction of the control or patient sample}}{\textbf{Extinction of calibrator 2}}$$

Specimens with antibody concentrations >18 U/ml above the mean concentrations of negative controls were considered as positive.

3.9 Data analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS, version 23.0) the statistical analysis of data was done using mean, standard deviation, chi-squareand the result were presented in form of tables and figures. A *p*-valuewas set less than 0.05 andconsidered significant results.

CHAPTER FOUR

RESULTS

CHAPTER IV
RESULTS

A total of 93 febrile Sudanese patient from Eastern Sudan (Kassala and Port Sudan) were included in this study, Their age range from 1 to 17 years old(mean6.8, SD± 4.13),Of these, 93 sample males 47 (50.5%) andfemales46 (49.5%) as in table (4.2). The majority of the patients were within the age group of 1-5 years (47.3%) as in table (4.1), Of the total 93 the duration of symptoms appeared among 3-7 days 84 (90.3%) as in table (4.3), While the majority of them from Kassala 69 (74 %) and 24 (26%) from Port Sudan as in figure (4.1).

Out of 93 patients, there were 83 (89.2) positive for anti-DENV-antibodies and 10 (10.8) were negative as in table (4.5).

Concerning age group and out of positive Anti- DENV- IgM, the majority of positive there were anti-DENV-antibodies were belong to age group 1-5 years 36 (43.3%), 32 (38.6%) in the age group 6-11 years, and all age groups 12-17 years were positive for anti-DENV IgM antibodies. There was an insignificant association between age group and Dengue fever (*P*-value = 0.075). As shown in table (4.6).In regard to gender, there were 42 (50.6%) male, 41 (49.4%) in females were positive for anti- DENV- IgM Abs. There was in significant association between gender and anti-DENV-antibodies (*P*.value= 0.97) as shown in table (4.7). On average, 74 (89.2%) of the 83 patients with anti-DENV antibodies had symptoms lasting from 3-7 days, while 9 patients (9.8%) had symptoms lasting more than 7 days. An insignificant association was found between anti-DENV-antibodies and symptoms' duration (*P*.value=0.65).as in table (4.8).Among the positive patients, 22 (26.5%) were from Port Sudan, while 61 (74.5%) were from Kassala. The residence group was not significantly associated with anti-DENV antibodies (*P*.value = 0.23) as in table (4.9).

Table (4.1): Distribution of age groups among the study population

Age groups	Frequency	Percentage
1-5 years	44	47.3
6-11 years	34	36.6
12-17 years	15	16.1
Total	93	100

Table (4.2): Distribution of gender among the study population

Gender	Frequency	Percentage
Male	47	50.5
Female	46	49.5
Total	93	100

Table (4.3): Duration of symptom among the study population

Duration of Symptom	Frequency	Percentage
3-7 days	84	90.3
More than 7 days	9	9.7
Total	93	100

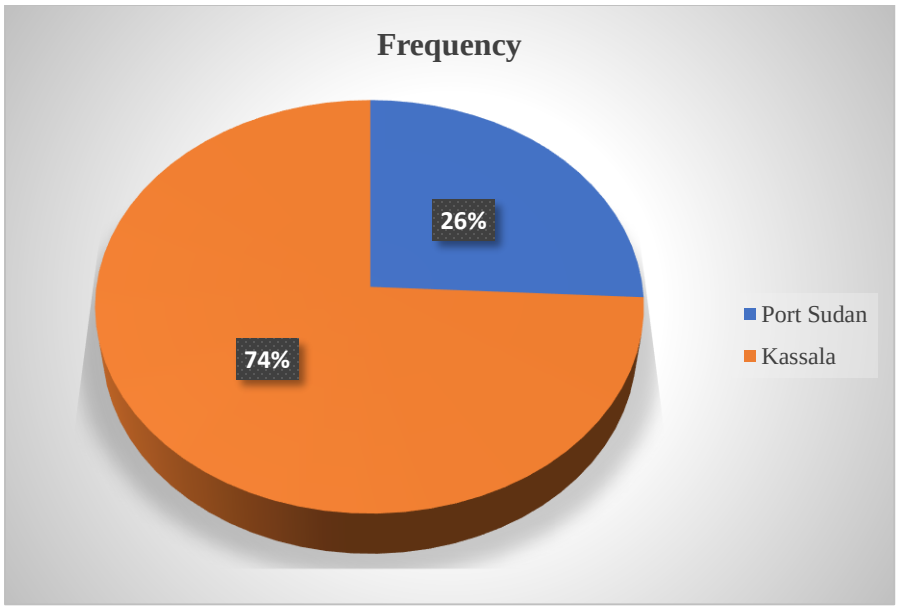

Fig (4.1): Distribution of residence among the study population

Table (4.4): Distribution of symptoms among the study population

Symptoms		Frequency	Percentage
Fever	Yes	93	100%
	no	0	0%
Headache	Yes	83	89.2%
	No	10	10.8%
Vomiting	Yes	49	52.7%
	No	44	47.3%
Bleeding	Yes	15	16.1%
	No	78	83.9%
Convulsion	Yes	10	10.8%
	No	83	89.2%

Table (4.5): Frequency of anti-Dengue virus IgM antibodies among the study population

Anti- DENV- IgM Abs	Frequency	Percentage
Positive	83	89.2%
Negative	10	10.8%
Total	93	100%

Table (4.6): Association between age groups and anti-Dengue virus IgM antibodies among the study population

Age group/ Years	Anti- DENV- IgM Abs		Total	*P*.value
	Positive (N %)	Negative (No %)		
1-5 years	36 (43.3%)	8(8%)	44 (47.3%)	0.075
6-11 years	32 (38.6%)	2 (2%)	34 (36.6%)	
12-17 years	15 (18.1%)	0 (00)	15 (16.1%)	
Total	83 (100%)	10 (100%)	93 (100%)	

Concerning age group, there were 36 (81.1) in age group 1-5 years, 32 (94.2) in age group 6-11 years, 15 (100) in age group 12-17 years. There were in significant associations between age group and anti-DENV-IgM antibodies.

Table (4.7): Association between gender and anti-Dengue virus IgM antibodies among the study population

Gender	Anti- DENV- IgM Abs		Total	*P*.value
	Positive (N %)	Negative (No %)		
Male	42 (50.6%)	5(50%)	47 (56.6%)	0.97
Female	41(49.4%)	5(50%)	46 (54.4%)	
Total	83 (100%)	10 (100%)	93 (100%)	

Concerning gender group, there were 42 (89.4) in male group, 41 (89.1) in females group. There was in significant association between gender group and anti-DENV-antibodies.

Table (4.8): Association between duration of symptoms and anti-Dengue virus IgM antibodies among the study population

Duration of symptoms /day	Anti- DENV- IgM Abs		Total	*P*.value
	Positive (N %)	Negative (No %)		
3-7 days	74 (89.2%)	10 (100%)	84 (90.3%)	0.65
> 7days	9(10.8%)	0 (0%)	9 (9.7 %)	
Total	83 (100%)	10 (100%)	93 (100%)	

Concerning duration group, there were 74 (88) in 3-7days, 9 (100) in more than7days, There was in significant association between duration group and anti-DENV-antibodies.

Table (4.9): Association between residence and anti-Dengue virus IgM antibodies among the study population

Residences	Anti- DENV- IgM Abs		Total	*P*.value
	Positive (N %)	Negative (No %)		
Port Sudan	22 (26.5%)	2 (2%)	24 (29%)	0.23
Kassala	61 (74.5%)	8 (8%)	69 (71%)	
Total	83 (100%)	10 (10.8%)	93 (100%)	

Concerning residence group, there were 22 (91.7) in Port Sudan group, 61 (88.4) in Kassala group, There was in significant association between residence group and anti-DENV-antibodies.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

CHAPTHERV

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Dengue is an important emerging disease of the tropical and subtropical regions of the world. In common with other vector-borne diseases, dengue requires conductive predisposing conditions for endemicity and outbreaks (Hassanain *et al.*, 2010).

In this study, the frequency rate of the dengue virus was 89.2%, and most febrile patients were younger than 5 years (47.3%), with most of them experiencing symptoms for 3-7 days (90.3%).Since the infection was an outbreak in Red Sea and Kassala States at the time of the study conducted and because the symptoms of dengue fever appear between 2-7 days. This finding agreed with previous epidemiological surveys; high prevalence rates (71.7%) of DENV seropositivity were reported among residents of Kassala by Abdalla *et al*., (2015), which was significantly high among the population of the state because newly constructed irrigation projects and agricultural schemes which provides a suitable habitat for the survival of *Aedes* vectors in this region. In addition, the heavy rainfall in as a potential risk factor for contracting DENV infection.Localities were identified as potential risk factors for contracting DENV infection by Eldigail et al (2018) in El-Gadarif State. Residents of El-Gadarif State have a significantly high prevalence of DENV antibodies (47.6%). Lower prevalence was determined by Elduma and Osman, (2014)who found that the prevalence rate was (12.8%) among pregnant women in the Red Sea State of eastern Sudan.Also lower prevalence rate 9.4% was reported by Soghaier *et al*, (2015) in Kassala State.Differ finding was stated by Who(2019) stated that there are 3823 cases including 11 associated deaths, giving a case fatality rate of 0.28%. The attack rate was 4.75 per 10 000 population. Among all reported cases, 46% are female and 70.8% are older than 15 years of age. It is possible that the discrepancy in our study is due to the fact that this period is an outbreak time for dengue fever infections, as well as the small sample size and cross-sectional nature of the study.

In the current study the gender has no significant association with DENV seropositivity (*P*. value 0.97), and both sexes are equally susceptible to infection with DENV.Relatively similar result was observed by Shah *et al*,(2012) in Western Terai Region of Nepal that reported, the Dengue positive cases were higher in females (10.9 %) than males (9.0 %),Also, Garg *et al*,(2011)in India found that 48.3% of suspected cases were males (285/590) and51.7% were females

(305/590)with significant difference(*P*.value=0.039).Neither dengue fever nor its surveillance or diagnosis are widely or consistently implemented in Sudan. As well, there are many acute febrile diseases with an unknown cause, especially in urban areas, of which malaria is the most likely cause.

5.2 Conclusion

This study concluded that:

The frequency of Dengue fever among febrile patients is significantly higher in Eastern Sudan, because during that time Red Sea and Kassala States experiencing epidemics of fever. This study found no association between age, gender, residence, duration of symptoms and dengue virus infection.

5.3 Recommendations

This study recommended that:

Further study should be conducted in the future to overcome the limitation of the study.

Febrile patients in Eastern Sudan should be routinely investigated for dengue fever.

Mosquito breeding area should be eradicated.

Avoiding mosquito biting(use insect repellents, wearing the right clothing and mosquito net)

As additional risk factors for dengue infection, household density and window screens should be incorporated into a future study plan.

Increasing awareness among residents in Eastern Sudan about the dengue fever infection and increasing awareness about protective measures needed to reduce these infections

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Appendices

Appendix1

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Serofrequency of Dengue Virus Infection among Febrile Patients among inEastern Sudan

ID……..

Sex:

Male: ☐ Female: ☐

Age: ……years

Residence:

Port Sudan: ☐ Kassala: ☐ Other ☐

Specify……Duration of stay in Port Sudan ☐

Duration /Appearance of symptoms:

3-7 ☐ 7-14 ☐

Signs and symptoms:

A-Mild symptoms:

Fever: ☐ Headache: ☐ Muscle and joint pain: ☐

Neck stiffness: ☐

B-Sever symptoms:

Bleeding: ☐ Confusion: ☐

Seizures-shock ☐ Coma: ☐