DENGUE VIRUS INFECTION:
CLINICAL AND LABORATORY CHARACTERISTICS; AND OUTCOMES OF PATIENTS ADMITTED DUE TO DENGUE IN MOMBASA-KENYA
A MULTICENTER RETROSPECTIVE STUDY

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H58/10969/2018

A Dissertation Submitted in Partial Fulfillment for the Award of Master of Medicine in Internal Medicine,
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STUDENT'S DECLARATION

This dissertation is my own original work and has not been presented for a degree at any other University.

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LIST OF ABBREVIATIONS

AKI - Acute Kidney Injury

AKHM - Aga-Khan Hospital Mombasa

ARDs - Acute Respiratory Distress

APTT - Activated Partial Thromboplastin Time

PT - Prothrombin Time

ADE - Antibody-Dependent Enhancement

ALT - Alanine Transaminases

AST - Aspartate Transaminases

BNP - B Natriuretic Peptide

BM - Bone Marrow

CFR - Case Fatality Rate

DF - Dengue Fever

DSS - Dengue Shock Syndrome

DHF - Dengue Hemorrhagic Fever

DM - Diabetes Mellitus

DFWS - Dengue Fever Warning Signs

DENV - Dengue Virus

DVI - Dengue Virus Infection

ESRD - End-Stage Renal Disease

EDS - Expanded Dengue Syndrome

ECG - Electrocardiogram

GCS - Glasgow Coma Scale

INR - International Normalized Ratio

ITP - Idiopathic Thrombocytopenic Purpura

IgM - Immunoglobulin M

IgG - Immunoglobulin G

IFN - Interferon

MODs - Multiorgan Dysfunction

PCR - Polymerase Chain Reaction

PRBCs - Packed Red Blood Cells

SCM - Sub-coastal Margin

ABSTRACT

Background: Dengue infection is an acute vector-borne viral disease. The annual incidence is estimated at 390 million infections per year, with approximately 20,000 annual deaths worldwide. Clinical and laboratory characteristics have demonstrated geographical and regional variation. The clinical spectrum of the disease is heterogenous. Dengue poses a diagnostic challenge, partly due to its atypical presentation and overlap of symptoms with other febrile illness, especially in endemic regions with limitation of rapid diagnostic kits. Prolonged hospitalization, cost of care, morbidities and mortalities related to dengue has imposed substantial economic, social, and personal burden. This retrospective study describes the clinical and laboratory characteristics, treatment modalities, disease severity and complications among patients admitted due to dengue infection.

Objectives: To determine the clinical and laboratory characteristics and outcomes of patients admitted with dengue infection between February 2018 and January 2020 in three selected hospitals at the coastal city of Mombasa, Kenya.

Methods: This was a multicenter retrospective descriptive study. We included consecutive patients admitted due to laboratory confirmed dengue infection, from February 2018 to January 2020. Clinical and laboratory characteristics and socio-demographics data were extracted from patients' files and recorded into a study proforma. Data on dengue-related complications, length of hospital stay, and mortality during hospitalization period were summarized.

Results: Out of 601 dengue cases, 319 (53.1%) were males. The mean age was 36.2 years, with 65% of the study participants between 13-40 years. Average duration of the hospital stay was 4 days. Approximately 90% of the patients were admitted with non-severe dengue, while 26.9% had evidence of warning signs. The most common clinical presentation followed by fever (92.3%) were myalgia 467 (77.7%), headaches 439 (73.0%) and retro-orbital pains 112 (18.6%). Among the clinical warning signs, mild muco-cutaneous bleeding was recorded in 62 patients. The common hematological findings were thrombocytopenia in 441 (73.3%), followed by leucopenia in 366 (60.8%), and hemoconcentration in 76 (12.6%) of the cases. Mild to moderate elevation of AST in 247 (43.1%) and ALT 177 (30.8%) were the commonest biochemical findings. Sixty-six (10.9%) patients presented with severe dengue. AKI was the commonest complication observed in 37 (6.3%), followed by myocarditis 11 (2.9%), DSS 10 (1.7%) CNS dengue 8 (1.4%), ARDS 8 (1.4%), severe hepatitis 6 (1.0%) and severe hemorrhage 4 (1.0%). Among the patients with severe dengue, four died.

1.0 CHAPTER ONE: INTRODUCTION

Dengue virus infection is an acute vector-borne viral disease caused by one of the four antigenically distinct dengue virus serotypes, of the genus Flavivirus (1). Among the serotypes, the commonest are DENV1, DENV2, DENV3, and DENV4, transmitted by mosquito Aedes aegypti and less commonly Aedes albopictus (2)(3). The Flavivirus genus also includes organisms that cause yellow fever, Zika virus disease and West Nile fever.

The incidence of the infection has increasingly grown in recent decades. Both asymptomatic and mild manifestations are common, and many patients are misdiagnosed as other acute febrile illness (4). Before 1970, only nine countries had experienced dengue epidemics; however, the disease is now endemic in more than 128 countries worldwide (5). The annual incidence of dengue is estimated to be 390 million infections per year, of which 96 million develop clinical manifestation of varying severity (6). According to World Health Organization (WHO), 2.5-3.6 billion people are at risk of dengue infection. Dengue infection is estimated to cause about 20,000 deaths annually with 265 disability-adjusted life year (DALYS) per million population annually (7).

There are wide variations of dengue incidence in the world. In Africa, the geographical and regional variations have also been observed (8). According to 2019 meta-analysis study, western Africa was noted to have a higher prevalence than eastern and central African countries (8). The geographical heterogenicity in prevalence may be due to difference in the human immunity across the African continent, geospatial variation of DENV vectors, and weaker health care systems including misdiagnosis and under-reporting of dengue infection. More so, regional variations have also been noted in several countries in Africa, including Nigeria (9).

The WHO 2009 revised classification categorized dengue infection according to severity into DF without warning signs, dengue fever with warning signs (DFWS) and severe dengue (SD) (11)(12). Non-specific clinical manifestation, including high fever, headaches, myalgia, retro-orbital pain, joint pains, and maculopapular rash occur during the febrile phase (10). Warning signs (WS) include abdominal tenderness, persistent vomiting, hepatomegaly, clinical fluid accumulation, muco-cutaneous bleeding, and rising hematocrit levels concurrent with thrombocytopenia (11)(12). SD may lead to severe hemorrhage, circulatory failure and multiple end-organ injury (10)(11).

The clinical, laboratory characteristics and outcomes of dengue infection show geographical and regional variations often depending on the viral and host factors (13). Host factors such as extremes of age, non-blacks, co morbidities, and secondary dengue infection are often associated with a higher risk of SD. Viral factors such as DENV 2 serotype and infection with multiple DENV serotypes predisposes to severe dengue. SD may result in prolonged hospital admission, intensive care (ICU) admissions , repeated blood transfusions, long term morbidities associated with disabilities, poor quality of life, high health costs, inability to work and mortalities (10).

The management of dengue infection presents a significant challenge especially in resource-limited countries due to inadequate diagnostic laboratory kits, atypical presentations of DENV infection, lack of vaccines and lack of effective vector control programs (2)(14). Despite the recent development of rapid laboratory kits for testing DENV; their availability is still limited. Most diagnoses in low-resource settings are based on clinical and epidemiological criteria (15). The infection has imposed substantial economic burden on both patients and health care systems in terms of hospital stay, morbidities and mortality (16)(17).

No local data exists regarding dengue-related clinical manifestation and outcomes, and the worldwide variations has not been documented in Kenya. This study will provide knowledge on the clinical manifestation and outcomes of patients with dengue infection, bridging the gap in knowledge and forming a basis for further research to improve patient's outcomes.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Epidemiology

2.1.1 Global Incidence and Geographical Variation

The annual incidence of dengue infection has increased dramatically by over fifteen-fold over the last two decades, with approximately 3.5 billion people currently at risk (17). Worldwide, the annual incidence of dengue infection is estimated at 390 million infections of which 96 million manifests clinically (17).

In 2019, it was the highest number of dengue infection cases ever to be reported. The American region alone confirmed 3.1 million dengue cases, with approximately 25000 cases categorized as SD according to WHO 2009 classification (18). Asia and Europe also reported high numbers of cases in many countries.

Outbreaks of the infection have also been confirmed and reported in more than 20 countries in sub-Saharan Africa, including western, central, eastern and southern Africa (9). Geographical and regional variations have been demonstrated, with west African countries having a higher prevalence rate than east and central Africa (9). The Immunoglobulin G (IgG) seroprevalence of dengue in eastern Africa was 3.6%, while the ribonucleic acid (RNA) prevalence was 5.3% (9). The relatively low seroprevalence rate observed in the east Africa countries could be indicative of misdiagnosis, under-recognition and under-reporting.

2.1.2 Global Burden of Dengue

Dengue is estimated to cause about 20,000 deaths annually with 265 DALYS per million annually (7). The case fatality rate (CFR) related to dengue infection demonstrates geographical and regional variation, with some countries reporting as high as 10-15%, and <1% in others (19)(20). Dengue-related prolonged hospitalization, cost of care, morbidity, and mortalities has also imposed substantial economic, social and personal burden, especially in low-income countries where the infection is becoming endemic.

2.1.3 Dengue Virus Infection in Kenya

Dengue cases has been documented in 2011 and 2013 at the north eastern town of Mandera, affecting 1300 and 190 individuals, respectively (21).

In Kenya, regional variation of dengue has also been observed. In a study conducted by Kenya AIDS Indicator Survey (KAIS) in 2007, the overall seroprevalence of dengue in Kenya was 12%, with higher prevalence in Coast Province at 60.6%, and the lowest prevalence in western province at 2.4%. Another study involving 1500 participants in Mombasa, 13% of the

individuals had evidence of recent dengue infection (22). According to a survey conducted by DDSR in the coastal city of Mombasa involving 148 blood samples, 56% of the participants tested positive for DENV (23). According to an entomological study conducted in Mombasa between 2013 and 2014, the risk of dengue transmission was high as evidenced by Breteau index (BI) of 270.1, container index of 31% and an overall house index of 22% (23).

2.1.4 Patterns of Transmission

Aedes aegypti are the principal vector, and are widely distributed in tropical and subtropical areas from latitude 35* South to 45* North (25). They are essential in maintaining both transmission through a human-mosquito human cycle (24).

The local and worldwide incidence and geographical distribution of dengue have been increasing due to human behaviors like population growth, poor urbanization with overcrowding, increased human movements, leading to an increased density of susceptible hosts (25). Other factors include increased vector density in tropical and subtropical regions associated with increased seasonal rainfall, effects of global climate change, poor sanitation with open water storage containers near homes and lack of effective mosquito control (24)(25).

2.2 WHO 2009 Classification of Dengue Infection

In 2009, the WHO published the revised classification scheme of categorizing dengue infection according to the severity. The classification has a higher sensitivity of detecting disease severity and enabling early recognition of warning signs (26). The WHO issued an additional document in 2011 and 2012 to include the atypical dengue infection presentations (27).

Table 1: WHO 2009 classification

Dengue Fever (DF)	DF with Warning sign (DFWS)	Severe dengue (SD)
Fever plus 2 or more of: - Headaches - Retro-orbital pain - Myalgia/arthralgia - Rash - Leucopenia - Positive tourniquet Laboratory-confirmed	DF plus any of: - ascites/or pleural effusion - hepatomegaly>2cm - mucosal bleed - hematocrit >20% - thrombocytopenia - severe abdominal pain	DF plus any of: - dengue shock - ARDs - Massive bleed - Severe organ damage (AKI, CNS, hepatitis, myocarditis)

2.3 Clinical Manifestations and Laboratory Derangements

The clinical presentation of the infection ranges from a mild self-limiting febrile illness to severe and life-threatening disease. In 2009, WHO classified dengue according to severity into DF, DFWS, and SD (11)(30). Symptomatic dengue is defined into three phases; febrile phase, followed by a critical phase, and finally, a recovery phase. Often, dengue-related complications appear during the critical phase (11)(30).

2.3.1 Dengue Fever- DF

Sudden onset high-grade fever > 38.5*C characterizes the febrile phase, which typically lasts for three to seven days with most patients recovering without complications (31)(32). This is usually accompanied by headaches, myalgia, headaches, vomiting, arthralgia and a macular rash (31)(32). In a prospective study involving 3,926 patients; headaches, eye pain and joint pains occurred in 60 to 70% of cases (33). Younger age and blacks are reported to have a higher prevalence of myalgia, arthralgia and headaches (34).

A maculopapular rash occurs in approximately 50% of the cases, typically two to five days after the onset fever (33). However, in a study done in Burkina Faso, the rash was noted in only 12.8% of the dengue confirmed cases (35). According to a Dar-salaam study, lower prevalence of rash was noted in blacks at 4.2% compared to non-blacks at 47% (34). In approximately 5% of cases, a biphasic saddleback fever curve is seen which typically remits and then recurs one to two days later (36).

Leucopenia due to bone marrow suppression is common during the febrile phase and could be an early predictor of severe disease. In one study, leucopenia and lymphopenia was noted in around 68% of dengue patients. However, according to a study done in Dar-salaam, only 33% of the dengue confirmed cases presented with leucopenia of < 3.5 (34). In one study, monocytosis was demonstrated in approximately 60-70% of the dengue cases (37). Significant monocytosis is commonly observed in SD which might be due to viral invasion and multiplication on the dermatological cells, including monocytes (38).

2.3.2 DF with Warning Signs- DFWS

To assist clinicians in early detection of severe disease progression, the 2009 WHO dengue guidelines recommended the use of warning signs as early indicators of plasma leakage (26)(27). Severe plasma leakage especially during the febrile phase, indicates high risk of disease progression, thus of great clinical value (26)(27)(17). However, their utility in predicting other dengue related outcomes including prolonged hospitalization and mortalities have shown mixed results in several studies.

The critical phase occurs typically around defervescence and lasts for about 24 to 48 hours (39). During the febrile and critical phases, laboratory parameters change with each day with subsequent normalization during the recovery phase (40).

Among the warning signs, persistent vomiting and severe abdominal pain have been reported to be the most frequent warning signs associated with SD. However, in one study, persistent lethargy was noted in most patients with SD and dengue-related death (41)(42). Other warning signs observed during the critical phase include; clinical fluid accumulation, mucosal bleeding, liver enlargement > 2 cm, rising hematocrit > 20 % from the baseline, and rapid drop of platelet count (43).

2.3.2.1 Rising Hematocrit

The rising hematocrit observed in dengue is due to plasma leakage beginning in the febrile phase and further pronounced during the critical stage (34)**.** Plasma leakage is thought to be secondary to endothelial cell dysfunction caused by pro-inflammatory markers such as tumor necrosis factor-alpha (TNF-alpha) and antibodies to DENV nonstructural protein 1 (NS1) (45). A rise of hematocrit by more than 20% from baseline as determined by serial hematocrit measurements has been associated with significant vascular leakage (44).

Occasionally, patients with SD have significantly higher hematocrit levels compared to patients with DF without warning signs. In a study conducted in Ethiopia, hemoconcentration of > 44%

was noted in 6.9% of the dengue cases (46). However, other studies have noted higher prevalence of up to 50%.

Several studies have reported an association between rising hematocrit and subsequent development of severe dengue. However, in a study conducted in Dar-salaam, no association was established (44). Hemoconcentration is widely believed to be closely associated with a degree of vascular leakage, rather than disease severity (34).

2.3.2.2 Thrombocytopenia

Varying degrees of thrombocytopenia is common during the febrile phase, followed by rapid improvement at the recovery phase (44). Often, moderate to severe thrombocytopenia has been demonstrated during the critical phase, occasionally reaching nadir count of < 20,000 cell/m* (47)(44)(48). In a study conducted in Ethiopia, thrombocytopenia was the most commonest hematological derangement observed, occurring in-more than half of the dengue cases (46).

Thrombocytopenia is initially thought to be due to bone-marrow suppression, however, immune-mediated platelet destruction and platelet adhesion to vascular endothelium are believed to cause progressive thrombocytopenia later during the critical phase (49)(50)(51)(52). Thus, a steady decline of platelet count is thought to correlate more closely with the severity of vascular leakage, rather than hemorrhagic tendency.

Several studies have reported that thrombocytopenia correlates poorly with bleeding manifestation (53)(54). However, some studies have reported a strong association between severe thrombocytopenia < 10 (rapid drop) and hemorrhagic manifestations, as seen in SD (43).

2.3.2.3 Mucosal Bleeding

Several studies have established that minor muco-cutaneous bleeding which may be observed in DFWS include a positive tourniquet test, gum bleeding, epistaxis, conjunctival bleeds, petechiae, and ecchymoses (55)(56)(57). Two Cuban studies reported predominant cutaneous bleeding such as petechiae or ecchymoses in approximately half of the dengue cases (58)(59). Other less frequent manifestation observed include gum bleeding and epistaxis 10% (57)(55)(56). Other studies have reported predominant mucosal bleeding compared to cutaneous bleeding (34).

According to a study done in Dar-salaam, mucosal bleeding was reported in 18.8% of dengue cases; however, it was not associated with the severity of the disease (34). In contrast, other studies have reported an association between mucosal bleeding and the severity of the disease.

2.3.2.4 Clinical Fluid Accumulation and Hepatomegaly

The frequency of hepatomegaly in dengue patients may range from 4%-52%. In one study, hepatomegaly was observed in only 22% of the dengue cases (46). The variation in the

prevalence of hepatomegaly might be explained by the difference of the virus serotype and genotype and their hepatotropic effects.

Clinical fluid accumulation related to dengue may range from pleural effusion, ascites, or gallbladder wall thickening. In one study, it was established that pleural effusion was the commonest at 60%, followed by ascites 53% and gallbladder thickening at 43%(61). However, in another study conducted in India involving 422 patients, pleural effusion was observed in only 4.2% of the cases, and none had ascites (62). According to a study done in Dar-salaam, clinical fluid accumulation was not associated with the development of severe dengue (44).

2.3.3 Severe Dengue

During the critical phase, the patient may progress to sere plasma leakage, characterized by systemic vascular shock, severe bleeding, and severe organ impairment including the brain, heart, liver, and kidneys (55).

2.3.3.1 Dengue Shock

Initially, narrow pulse pressure of < 20mmHg may occur, which might be secondary to physiological compensation. Initially, the systolic blood pressures (SBP) may be normal or elevated, however, blood pressure may fall rapidly accompanied by irreversible shock despite adequate resuscitation (44). In Sub-Saharan Africa, a study in Dar-salaam reported a 4.2% prevalence of SD. Out of the twenty patients classified as SD, seven presented with low SBP of < 90 mmHg, five patients presented with narrow pulse pressures (NPP), and two patients presented with both. Additionally, tachycardia and cold extremities may also be noted (34). In contrast, higher prevalence of hypotension and NPP have been demonstrated in other studies.

2.3.3.2 Massive Bleeding and Coagulopathy

Hemorrhagic clinical spectrum in patients with dengue may range from minor muco-cutaneous bleeding such as epistaxis and gum bleeding to major bleeding. Significant bleeding especially from GI tract or the Genitourinary system is often associated with profound shock. In one study involving 369 dengue patients, massive bleeding was observed in 149 cases. 59% of the patients presented with gastrointestinal (GIT) bleeding, while hematuria and hemoptysis were less frequently demonstrated.

Severe plasma leakage with resultant excessive intravascular volume depletion may lead to the abnormal activation of the coagulation system (63)(59)(64)(44). Transient abnormalities of the coagulation profile are not uncommon in patients with SD (65)(52)(65)(64)(66). These abnormalities are widely considered to be associated with massive bleeding in SD; however,

some studies have reported their association with severity of plasma leakage and not massive hemorrhage (54).

Massive GIT bleeding has been frequently associated with dengue-related prolonged hospitalizations and mortalities (67)(60) however, in another study, no association was established (34).

2.3.4 Severe Organ Impairment

Atypical presentations of dengue including severe hepatitis, neurological, renal, cardiac, gastrointestinal, and other isolated organ involvement are increasingly reported in severe dengue, and occasionally in classical DF patients without evidence of plasma leakage.

2.3.4.1 Neurological

Dengue-related neurological complications occur in approximately 1%-4% of cases. In a Vietnamese study, the most frequent neurological manifestation reported was reduced level consciousness, while a few patients presented with encephalitic manifestations such as fever, headache, convulsions or lethargy (68). However, a few patients may not present with any neurological symptoms (69). In such cases, the diagnosis of dengue-associated central nervous system (CNS) disease may be supported by virus detection in culture or PCR and serological analysis of cerebrospinal fluid (68). The CNS manifestation are thought to be secondary to immune-mediated mechanisms; however, direct viral neurotropism has also been demonstrated.

2.3.4.2 Liver

Hepatic manifestations may range from asymptomatic transaminitis to acute fulminant liver failure (70)(71). Complications such as severe bleeding, hepatorenal failure, encephalopathy, and metabolic acidosis may be observed in patients with severe dengue. Moderate to marked elevation of the aminotransferases has been reported in several studies, with aspartate transaminases (AST) more prominent than the rise of alanine transaminases (ALT), suggesting the non-liver sources of AST, including damaged monocytes and platelets (44)(36). According to a study done in Ethiopia, the elevation of AST and ALT levels were demonstrated in 45% and 17.5% respectively, in patients infected with dengue (46).

Acute liver failure has been attributed to ischemic hepatitis, although direct viral invasion and immune-mediated injury of the hepatocytes may be an alternative mechanism.

2.3.4.3 Cardiovascular

Dengue-related cardiac manifestations are not uncommon and might have been under-diagnosed and under-reported. Transient dengue myocarditis can vary from asymptomatic non-

specific electrocardiogram (ECG) abnormalities through to a more severe clinical manifestations (72)(73)(74)(75). In some studies, the ECG abnormalities were observed in 30-40% of dengue cases especially during the critical phase, and are usually transient and non-specific (75)(76). In one study, elevated troponins and/or brain natriuretic peptide was demonstrated in 15% of the dengue cases (74). Cardiomyopathies with resultant systolic and diastolic dysfunction have also been reported in several studies, with septal and right ventricular walls predominantly affected (75) (77). Although immune mediated mechanisms have been associated with dengue myocarditis, histologic findings at autopsy have demonstrated detection of DENV antigens in cardiomyocytes (74)(78).

2.3.4.4 Kidney

Acute kidney injury (AKI) has been reported in dengue infection, ranging from 0.9% to as high as 69.4% (79)(80). The wide variations in the prevalence of dengue-associated AKI might be due to the difference of AKI classification system implemented in different studies (81). AKI is frequently associated with dengue-related poor outcomes. According to studies, the prevalence of AKI has been observed in approximately 0.9%-69% of fatal dengue cases (80). Dengue related renal involvements might be due to several mechanism including, direct renal invasion by the virus, shock-induced tubular necrosis, rhabdomyolysis or glomerulonephritis (82)(48)(83)(84). In Africa, Getachew et al in Ethiopia, reported an elevation of serum creatinine levels and hypoproteinemia in 19.6% and 21.6% respectively in dengue infected patients (46).

2.4 Prognostic Factors

Dengue-related prolong hospitalization, ICU admissions, morbidities and mortalities carry a substantial economic, social and personal burden. This is even worse in middle to low-income countries where infection is becoming endemic. Prolonged hospital stay is not only associated with SD, but also in patients with non-severe dengue infection (85).

Previous contradicting data have been reported regarding the determinants and factors associated with poor, however, interaction between viral and host factors appear to play a key role. Viral factors including DENV type 2 serotype, multiple infecting dengue serotypes, and secondary dengue infection, are frequently associated with SD. On the other hand, host factors including white race, age >40 years, female gender, co-morbidities (such as diabetes, hypertension), late presentation to the hospital, presence of warning signs, massive bleeding, coagulopathy, dengue shock, and presence of end organ damage are widely believed to be associated with SD, prolong hospital stay and mortalities.

2.4.1 Viral Factors

Both the viral serotype and genotype determines the severity of dengue. Severe dengue is commonly associated with serotype DENV 2 (86). Infection with more than one dengue serotype has been associated with increased risk of SD, prolong hospitalization, and mortality. Secondary dengue infection has been associated with more prevalence of SD (86)(29), however, severe disease may be also seen in primary dengue in exceptional cases.

2.4.2 Host Factors

Several studies including in Cuba and Haiti have shown that severe dengue occurred more commonly in whites than blacks (87). This might be due to stronger immune response in terms of CD-4 memory cells proliferation and over production of Interferon-gamma observed in white people compared to blacks (88). However, higher overall mortality rates among blacks have also been demonstrated compared to non-blacks, which might be related barrier to access to a quality health care (34).

In a study conducted in Dar-salaam in 2018, severe dengue was found to be independently associated with non-black race and diabetes mellites (34). In the study, old age and secondary dengue infection were not associated with severe dengue (34). This might be explained by the fact that Tanzania has been exposed to only one DENV serotype i.e. DENV 3, thus minimal risk of antibody-dependent enhancement (89).

In another cross-sectional retrospective study; elevated alkaline phosphatase, prolonged international normalized ratio and multi-organ disease syndrome were independently associated with prolonged hospitalization. The CFR was 1.1%, and was associated with age > 40 years, secondary dengue infection, co-morbidities (such as diabetes), hematocrit>20%, respiratory failure and prolonged hospital stay (90). Older age has been associated with SD and prolong hospitalization which might be due to higher incidence of DSS and co-morbidities in such age groups.

In a study conducted at Aga-Khan University Hospital-Pakistan involving 532 dengue patients; one third of the cases (27.3%) had prolonged hospital stay of > 3 days. In the study, factors associated with prolonged hospitalization included AKI, coagulopathy (prolonged PT and APTT), and increased age of > 41.10 years. Mortality was 1.5%; high mortality was found in those with AKI, dengue associated hemorrhage, shock, coagulopathy, respiratory failure, and increased hospital stay (91).

2.5 Justification

The annual incidence of dengue infection has increased dramatically by more than 15-folds over the past two decades, with approximately 3.5 billion people currently at risk of infection. Sporadic outbreaks have been reported worldwide, including countries in the African continent.

In Kenya, higher seroprevalence rates of both recent and previous dengue infection has been documented in the coastal city of Mombasa compared to other regions of Kenya.

The WHO estimates approximately 20,000 annual deaths related to dengue, with additional 265 DALYS per million population annually. Dengue-related prolonged hospitalization, cost of care, morbidities and mortalities has imposed substantial economic, social, and personal burden, especially in low-income countries.

In Africa, the management of dengue poses a great challenge; geographical variation and atypical presentation, with lack of adequate diagnostic kits has contributed in under-recognition and underreporting of dengue in Africa (8). Limitations of currently available control strategies such as vaccines and pesticides have further led to increase in the burden of the disease. Although the non-specific symptoms of dengue may be similar to other febrile illness (10), some specific features maybe distinct especially in patients with warning signs and severe dengue (6)(26)(27).

There are only limited studies done on clinical characteristics and outcomes of dengue in the sub-Saharan Africa. These studies have also demonstrated significant geographical and regional variations in terms of clinical and laboratory characteristics features.

In our set-up, no local data exists. Our study emphasized to document the clinical and laboratory characteristics of patients admitted with dengue, enabling categorizing disease severity according to WHO 2009 guidelines and compare with available literature (26)(27).

The evidence generated is crucial for early diagnosis, improving patient triage and prompt management of dengue. This will help reduce morbidity, mortality and improve economic impact of dengue in terms of hospital stay (26)(27).

This information will be relevant in bridging the gap in knowledge, forming a basis for further research to improve patient outcomes. The information might also guide the policy makers in allocation of financial budget and adequate infrastructure including bed capacities in future epidemic seasons.

2.6 Research Question

What are the clinical and laboratory characteristics; and outcomes of patients admitted due to dengue virus infection in Mombasa-Kenya, between February 2018 and January 2020?

2.7 Study Objectives

2.7.1 Broad Objective

To determine the clinical and laboratory characteristics; and outcomes of patients admitted due to dengue virus infection in selected private hospitals in Mombasa between February 2018 and January 2020.

2.7.2 Primary Objectives

a) To describe the clinical characteristics of patients admitted with dengue infection in selected private hospitals in Mombasa between February 2018 and January 2020.

b) To describe the laboratory characteristics of patients admitted with dengue infection in selected private hospitals in Mombasa between February 2018 and January 2020.

c) To document the types of treatment modalities of patients admitted due to dengue infection in selected private hospitals in Mombasa between February 2018 and January 2020.

2.7.3 Secondary Objective

To determine the types and frequency of complications among patients admitted due dengue infection.

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Site

The coastal town of Mombasa is the second-largest city in Kenya. Health services in Mombasa are provided by both public and private hospitals. This study was conducted in three major private hospitals, i.e., The Mombasa hospital, The Aga-Khan, and The Premier hospitals, serving a cosmopolitan catchment population of approximately 500,000 residents.

The study area was selected based on the evidence of recurrent previous outbreaks and the availability of well-kept health records. High temperatures of up to 34*C, followed by rainy season, have contributed to an increase in the vector density. Furthermore, human behaviors, including, high numbers of tourists, unplanned rapid urbanization, and poor sanitation, have also increased disease transmission among the susceptible hosts.

All the three hospitals have well established 24-hour operational outpatient clinics, wards, and ICU facilities, with a combined total bed capacity of approximately 500 beds. All the study participants were initially attended by a medical officer and then reviewed by consultant physicians within 24 hours of admission.

3.2 Study Design

The study was a multicenter retrospective descriptive study to evaluate the clinical and laboratory characteristics, and outcomes of patients admitted due to dengue, an infection that is often sporadic and seasonal in Mombasa, Kenya.

3.3 Eligibility criteria

The study populations included all patients admitted due to laboratory confirmed dengue infection. Laboratory-confirmed dengue involved patients with a positive dengue NS1 antigen and/or dengue auto-antibodies IgM.

3.3.1 Inclusion Criteria

All files of patients above 13 years, admitted due to laboratory-confirmed dengue infection, between February 2018 and January 2020.

3.3.2 Exclusion Criteria

Records of patients having incomplete or insufficient medical records were excluded. Files with no data on variables of interest were not included. Files containing relevant socio-demographic and co-morbidity data, and information on clinical-laboratory characteristics, treatment modalities and complications were considered sufficient enough to describe severity of disease according to 2009 WHO dengue classification.

3.4 Sample Size Determination

Being an audit, all consecutive files of patients meeting the eligibility criteria were enrolled. However, a minimum sample size was calculated using Fishers Formula;

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

where **n**= Desired sample size

Z= value from standard normal distribution corresponding to desired confidence interval (Z=1.96 for 95% CI)

p=expected true proportion (estimated at 70.0%, in a prospective study involving 3,926 patients, headaches, eye pain and joint pains occurred in 60-70% of cases)

d= desired precision (0.05)

A minimum sample size of 323 patients was required for the study.

3.5 Sampling Technique

Using ICD A90/91, inpatients numbers of all dengue cases admitted at the three private hospitals during the aforementioned study period were retrieved. Being an audit, all consecutive files of patients aged 13 years and above, who were admitted due to laboratory-confirmed dengue infection during the study duration, and also meet the eligibility criteria were enrolled.

3.6 Research Tools

Study data was sourced from patient's medical records. The missing laboratory records were retrieved from the main laboratories. A data collection tool was used to collect variables of interest.

3.7 Data Collection

Variables of interest were extracted using the Data Collection Form. Socio-demographic and clinical data of interest were recorded into the study proforma. Hematological parameters such as leucocyte count, monocyte count, lymphocyte count, hemoglobin levels, hematocrit levels, platelet count, and creatinine levels at admission were recorded. The Highest (peak) and the lowest levels (nadir) of specific laboratory parameters such as; hematocrit level, total leucocyte count and its differentials, serum creatinine levels, AST and ALT during the hospitalization period were also recorded.

Treatment strategies such as critical care, dialysis, blood transfusion, and administration of drugs including antibiotics were also recorded. Dengue-related complications including severe organ targets, dengue shock, severe bleeding, and fatal cases during hospitalization were also recorded. Dengue severity was graded according WHO 2009 classification into non-severe dengue and SD.

3.8 Study Variables

-Dengue recurrence: Secondary dengue; as demonstrated by positive IgG dengue antibodies during the acute phase of disease (< 5 days of onset of symptoms), OR physician documentation.

-Chronic illness*:*

Known co-morbidities such as hypertension, and diabetes.

-Clinical characteristics: documented symptoms and warning signs of dengue infection by the medical officer or physician, during admission and hospitalization period.

-***Fever***

Defined as axillary temperature of > 38^0C.

-***Hypotension***

Defined as low systolic blood pressures of < 90 mmHg as per 1997 WHO dengue classification.

-***Narrow pulse pressure***: systolic and diastolic blood pressures differences of < 20 mmHg.

-***Tachycardia*:** peripheral pulse rate of >100 beats/minute.

-***Mild Muco-cutaneous bleed***: Documented history of gum bleeding, conjunctival bleeding, epistaxis, petechiae, or ecchymoses without need for blood transfusion.

-***Fluid accumulation***: Documented new onset ascites or pleural effusion as detected by either physical examination or imaging (CXR, CT scan, ultrasound).

-***Hepatomegaly***:

Liver enlargement > 2 cm below the SCM, as detected by either physical exam or imaging.

-Laboratory characteristics: Findings and interpretation of selected laboratory parameters performed during admission and hospitalization.

- ***Anemia:*** nadir hemoglobin levels (women < 12 g/dl and <13 g/dl for men)
- ***Leucopenia*:** lowest leucopenia count (< $4x10^{9/L}$)
- ***Lymphopenia:*** nadir lymphocyte count (< $1x10^{9/L)}$
-***Leukocytosis*:** highest leucocyte count (> 11 $x10^{9/L}$)
- ***Monocytosis***: highest monocyte count (> $0.9x10^{9/L)}$

-***Hemoconcentration*:** defined as increase in hematocrit level by > 15% from the baseline.

-***Thrombocytopenia***: defined as lowest platelet count (< $150x10^{9/L}$).

-**Treatment modalities***:* during hospitalization period.

- ***Critical care:*** defined as transfer to ICU/HDU for mechanical ventilation, non-invasive ventilation, or ionotropic support for patients with shock/severe organ targets.

-***Blood transfusion:*** transfusion of at least 1 unit of any of the blood products including packed red blood cells (PRBCs), platelet concentrates, FFP or whole blood.

-***Dialysis*:** Dengue-AKI necessitating at least 1 dialysis session in participants not previously on dialysis.

-**Complications**: unfavorable or unwanted outcomes of dengue infection at admission or during hospitalization period.

-***Dengue shock***: Tachycardia with narrow pulse pressure or hypotension.

-***Massive bleeding*:** As physician documentation, OR significant bleeding necessitating transfusion of at least 4 units of blood.

-***ARDS*:** New onset or respiratory symptoms and hypoxemia (PF < 300), with chest imaging demonstrating bilateral opacities not fully explained fluid overload or cardiac failure (Berlin definition)

-***CNS dengue***: Documented new onset altered mentation as defined by GCS of <14.

-***AKI*:**

Defined as an increase in serum creatinine levels by more than 26.5 micromole/L within 48 hours, OR, reduction of maximum serum creatinine by > 50% during the course of illness, OR, baseline creatinine estimation using MDRD formulae assuming normal GFR of 75ml/min/min, OR physician documentation.

-***Prolong hospital stay*:** defined as hospital stay > 3 days.

-***Mortality:*** all-cause mortality during hospitalization

-***Severe dengue***: SD was defined as dengue fever, plus any of the following;

- Dengue shock.
- Massive bleeding
- Severe organ impairment (CNS, AKI, Liver, ARDs, Heart),
- Death

3.9 Data Analysis

Data of interest was extracted using a Study Proforma. The study proforma had a serial number and was kept in a secured safe. Data was checked for completeness and free from error prior

to entry into Statistical Package for Social Sciences version 23.0. Continuous variables such as age were reported as mean with standard deviation (normal distribution), or median with interquartile range, if skewed. Data on clinical presentation, warning signs, laboratory abnormalities, type and frequency of treatment modality and complications were summarized as proportions with percentages. This data was presented in form of tables, pie-charts, and bar-graphs.

3.10 Ethical Considerations

Data collection was undertaken after ethical approval was obtained from the Kenyatta National Hospital/University of Nairobi Ethics and Research Review Committee, and Ethics committee of the respective hospitals. Absolute confidentiality was maintained throughout the study. The study results will only be released into the public domain after obtaining permission from institution where recruitments and procedures were done.

The primary researcher kept the study proforma, and the computer used for analysis, under lock and key. Confidentiality of the patients through unique numbers was ensured.

3.11 Study Feasibility

The study was conducted at the AGHM, The Mombasa Hospital and The Premier Hospital, which are among the largest and busiest hospitals at the coastal city of Mombasa. The relevant investigations were routinely done at admission and repeated within 24-72 hours after admission. 714 dengue patients were admitted during the study. Working on a schedule of 8 hours per day, we were able to review 16 files per day during 44 days of data collection, with two medical officers as research assistants.

4.0 CHAPTER FOUR: RESULTS

Seven hundred and fourteen patients were admitted from the three hospital facilities due to dengue infection in the 2-year period. Thirty-one files were missing and 82 had unclear diagnosis. A total of 601 files were enrolled in the study. Figure 1 summarizes selection of study cohort.

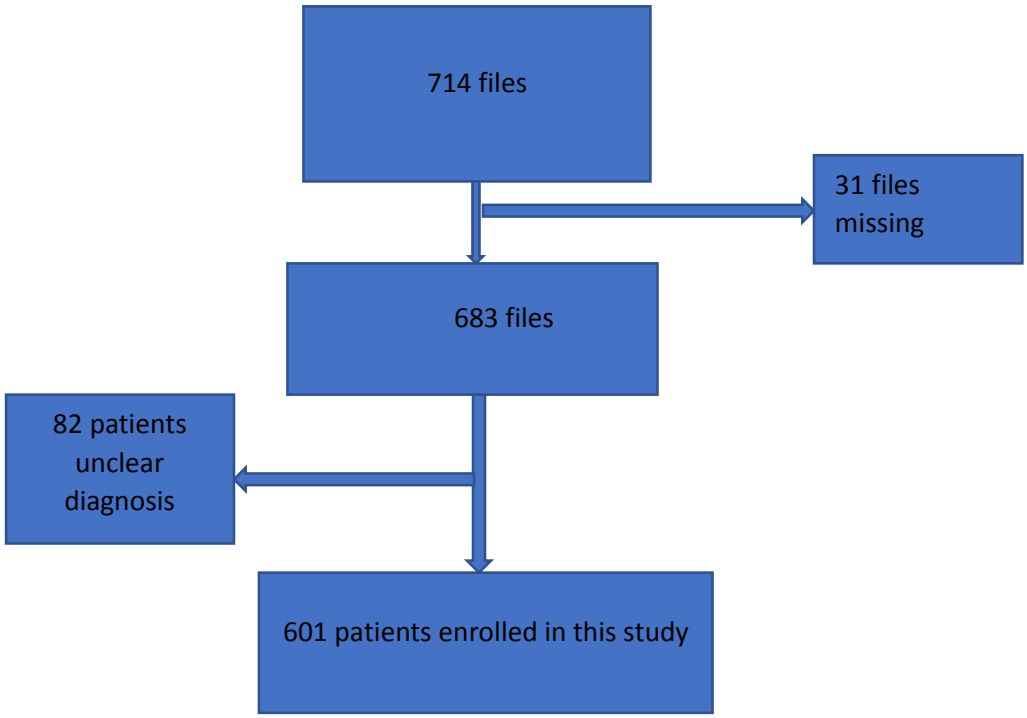

Figure 1:Flow diagram showing the selection of study cohort

4.1 Socio-Demographics Characteristics

Out of the 601 patients, 319 (53.1%) were males. The mean age of the respondents was 36.2 years (SD=12.7), of these, 389 (65%) were 40 years and below. The age distribution is represented in a bar graph as shown in Figure 2 below. Majority of the study participants (74.2%) had either primary or secondary education, while 131 (21.8%) patients had tertiary education. Only 24 (4.0%) patients had no education. There were 261 (43.4%) unemployed patients, while the majority of the employed were in business industry. Table 2 below summarizes the patient's socio-demographic characteristics.

Table 2:Socio-demographic characteristics of patients admitted due to dengue infection

Variable	Categories	Frequency *(n=601)*	Percent (%)
Age (years)	13-40	389	64.7
	40 and above	212	35.3
Gender			
	Male	319	53.1
	Female	282	46.9
Residence			
	Rural	335	55.8
	Urban	266	44.2
Education			
	Primary	223	37.1
	Secondary	223	37.1
	Tertiary	131	21.8
	None	24	4.0
Occupation			
	Employed	286	47.6
	Unemployed	261	43.4
	Self-employed	35	5.8
	Retired	19	3.2

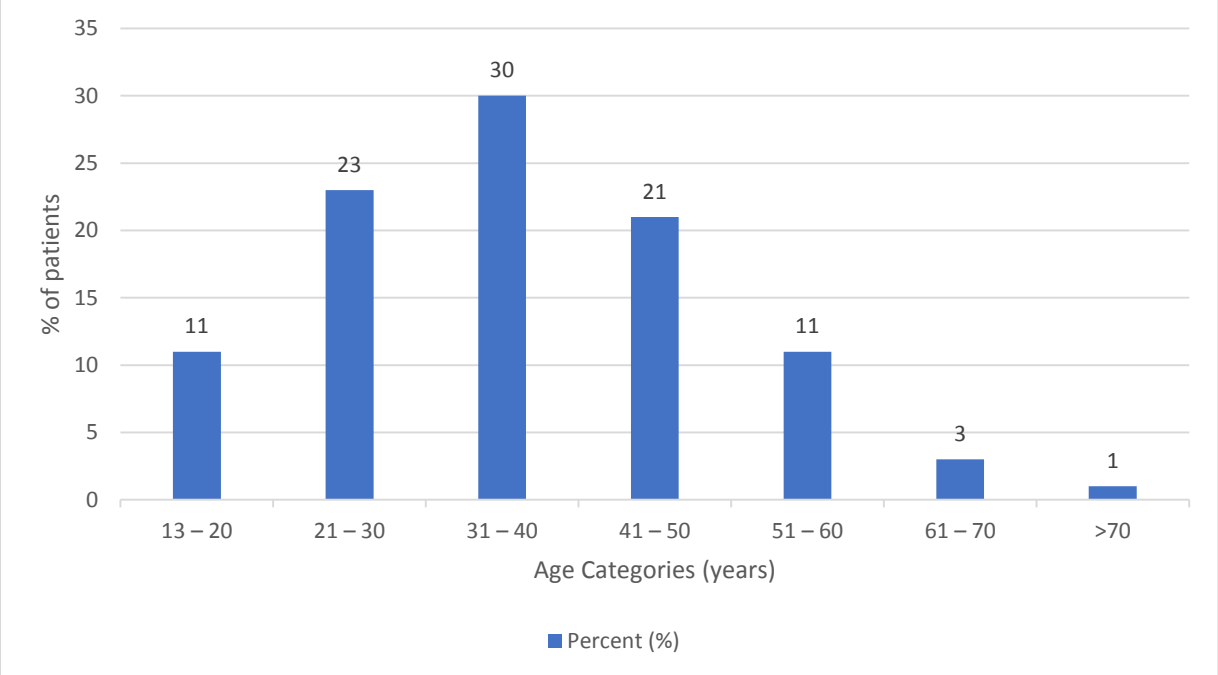

Figure 2:Age distribution

4.2 Dengue Recurrence and Co-morbidities

Most of the patients (87.7%) had primary dengue, while 74 (12.3%) of the cases were admitted with recurrent disease as shown in figure 3 below. Two hundred and ten patients (34.9%) had underlying conditions, while 24 patients (4.0%) had coinfection with malaria. Among the co-morbidities, hypertension was observed in 70 (11.6%) patients, while 44 (7.3%) of the study participants had diabetes. Table 4 summarizes patients' underlying conditions.

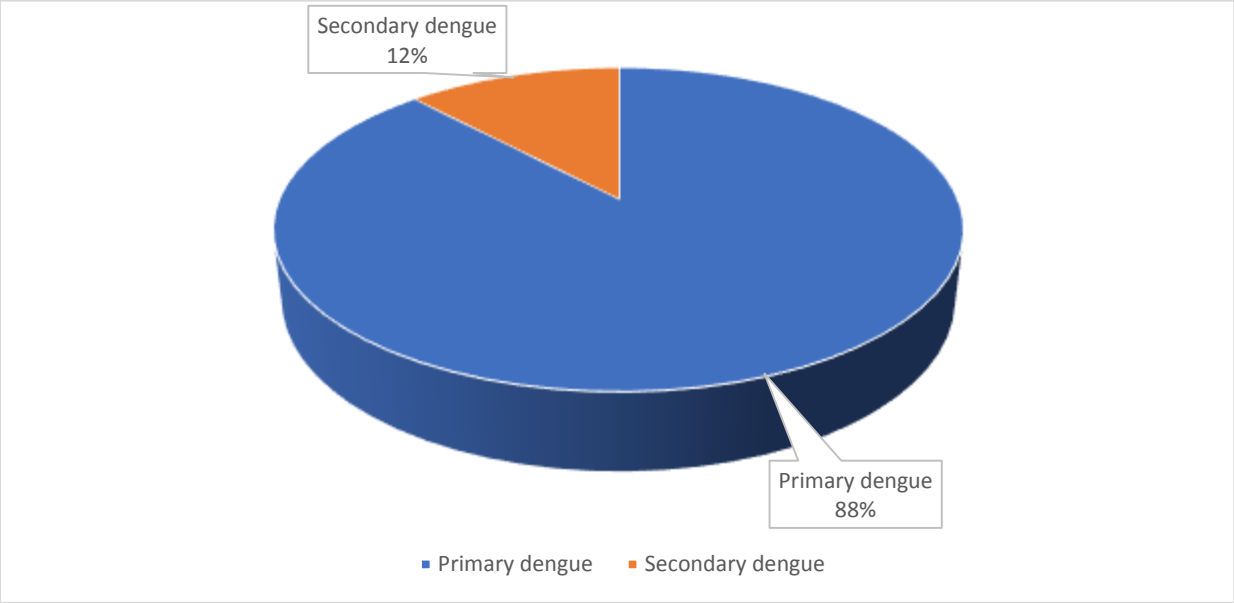

Figure 3:Disease recurrence

Table 3:Co-morbidities among the study participants

Variable	Frequency (n)	Percent (%)
Hypertension	70	11.6
Diabetes	44	7.3
Peptic ulcer disease	25	4.2
Asthma	15	2.5
Malignancy	12	2.0
Hematological disorders	10	1.7
Chronic kidney disease	5	0.8
Chronic liver disease	4	0.7
Other co-morbidities	25	4.2
Coinfection with malaria	24	4.0

4.3 Disease Severity

Out of the 601 enrolled, 535 (89.1%) were categorized as non-severe dengue, while 66 (10.9%) patients presented with severe dengue. One hundred and sixty-two study participants (26.9%) had evidence of warning signs, with only 36 (22.2%) progressing to severe disease. Figure 4 illustrates categorizing study participants according to disease severity.

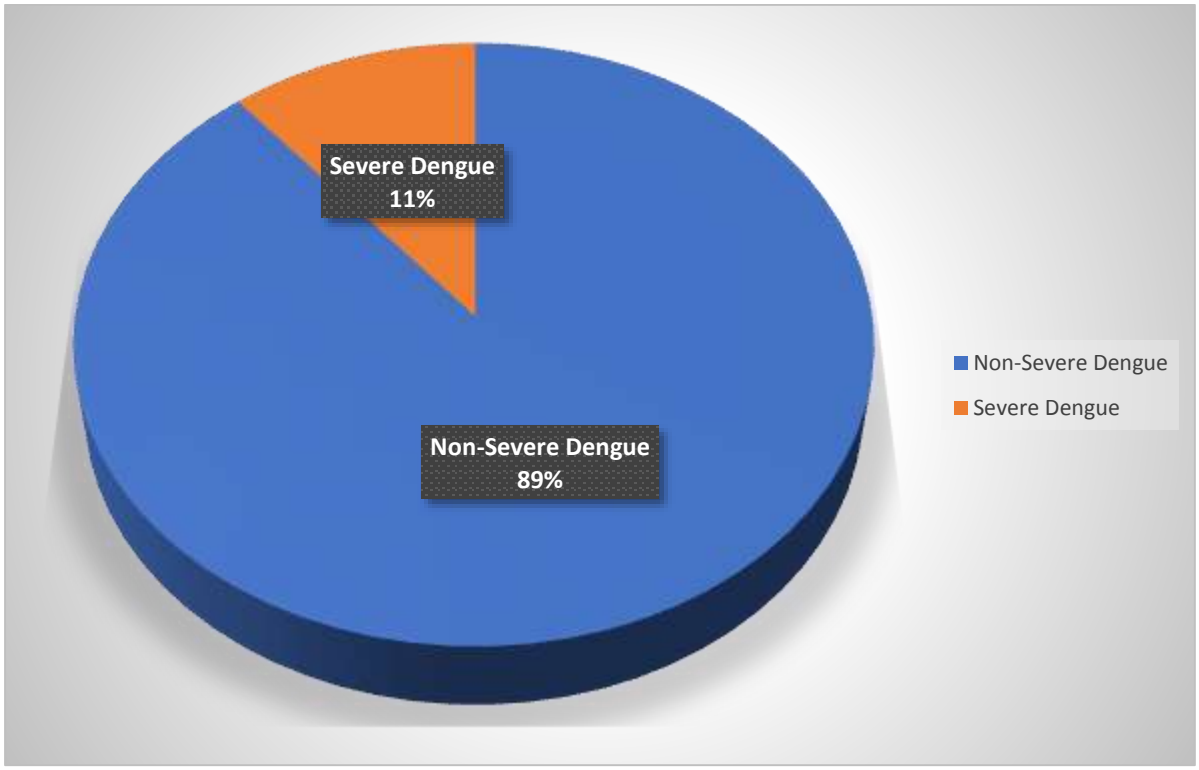

Figure 4:Severity of Dengue

4.4 Clinical Presentation

As shown in Table 4 below, the commonest presenting complaint was fever, documented in 555 (92.3%) of the patients, while 467 (77.7%) patients had myalgia or arthralgias. Four hundred and thirty-nine patients (73.0%) had headaches, while 112 (18.6%) of the study participants presented with retroorbital pains at admission. Dengue rash was evident in 89 cases (14.8%).

Among the clinical warning signs, mild muco-cutaneous bleeding was observed in 62 (10.9%) patients. Out of the 62 cases, a majority (62.0%) presented with mucosal bleeding as manifested by gum bleeding (3.4%), hematochezia (2.7%), melena (1.4%) or epistaxis (1.2%).

Clinical fluid accumulation was observed in 15 (2.8%) patients as manifested by pleural effusion in 13 (2.4%), and ascites in 11 (2.0%). Hepatomegaly was demonstrated in 10 (1.8%) patients. Table 5 below summarizes the warning signs as predictors of disease severity among patients admitted with dengue.

Table 4:Clinical Presentation at admission and during hospitalization

Variable	**Frequency *(n=)***	**Percent (%)**
Fever	555	92.3
Myalgia/arthralgia	467	77.7
Headache	439	73.0
Retro-orbital pains	112	18.6
Rash	89	14.8
Tachycardia	56	9.3
Systolic hypotension	21	3.4
Narrow pulse pressure	5	0.8

Table 5:Warning clinical signs during hospitalization

	Variable	**Frequency**	**Percent**
Muco-cutaneous bleeding*(n=566)*	Petechiae	25	4.4
	Gum bleeding	19	3.4
	Hematemesis	15	2.7
	Hematochezia	10	1.9
	Melena	8	1.4
	Epistaxis	7	1.2
	Others	5	0.9
Fluid accumulation*(n=545)*	Pleural effusion	13	2.4
	Ascites	11	2.0
Liver *(n=541)*	Hepatomegaly	10	1.8

4.5 Laboratory Parameters

The mean hemoglobin at admission was 13.6 g/dl, with a range of 12.7-14.6 g/dL. The highest and lowest means of hematocrit were 42.4% and 38.8% respectively, with a range of 36.2-45.8%. The mean platelet count at admission was $144x10^{9/L),}$ while the mean nadir platelet count during hospitalization was $107x10^{9/L),}$ with a range of 103-$182x10^{9/L}$. The mean WBC on admission was $4.9x10^{9/L)}$, with the lowest mean during hospitalization at 3.5 $x10^{9/L).}$

Table 6 describes the results of hematological parameters of the study participants at admission and during hospitalization.

4.6 Laboratory Derangements

The commonest laboratory derangement was thrombocytopenia as seen in 441 (73.9%) patients, followed by leucopenia documented in 366 (61.4%) participants. Among patients with thrombocytopenia, 273 (65.1%) presented with platelet counts of < $100x10^{9/L}$. A change of hematocrit by > 20% from baseline was evident in 76 (12.6%) study participants.

Out of 573 study participants, 246 (43.1%) patients recorded high aspartate aminotransferase (AST), while 177 (30.8%) had elevated alanine aminotransferase (ALT) during the hospitalization period. A majority of the patients had concentration higher than three to five times the upper limit of normal in AST (74.5%) and ALT (80.2%) as demonstrated in table 6 below.

Table 6: Hematological parameters at admission and during hospitalization

Variable	At Admission	Peak level (highest)	Lowest (nadir)
Hemoglobin (g/dl)			
Mean (SD)	13.6 (1.6)	14.1 (1.61)	13.2 (6.4)
Median (IQR)	13.7 (12.7-14.6)	14.1 (13.2-14.9)	13.0 (12.1-14.0)
HCT (%)			
Mean (SD)	40.5 (4.8)	42.4 (4.8)	38.8 (4.6)
Median (IQR)	40.2 (37.8-43.5)	42.6 (39.9-45.8)	39.0 (36.2-42.0)
Platelet count (10*9/L)			
Mean (SD)	144.1 (61.5)	161.3 (58.4)	107.9 (60.7)
Median (IQR)	142.0 (103.0-175.0)	157.0 (127.5-182.0)	102.0 (59.0-151.0)
Total WBC (10*9/L)			
Mean (SD)	4.9 (7.7)	7.3 (3.6)	3.5 (1.9)
Median (IQR)	4.1 (2.7-5.6)	6.3 (4.8-8.7)	3.2 (2.1-4.3)
Lymphocyte (10*9/L)			
Mean (SD)	1.5 (1.0)	2.5 (1.4)	1.1 (0.8)
Median (IQR)	1.3 (0.8-2.1)	2.3 (1.6-2.9)	0.8 (0.6-1.3)
Monocyte (10*9/L			
Mean (SD)	0.7 (0.6)	0.9 (0.7)	0.4 (0.3)
Median (IQR)	0.6 (0.3-0.8)	0.8 (0.5-1.1)	0.3 (0.2-0.5)
Neutrophil (10*9/L			
Mean (SD)	3.4 (2.4)	5.7 (3.3)	2.3 (1.8)
Median (IQR)	2.8 (1.6-4.7)	5.6 (3.4-6.8)	1.8 (1.0-3.1)

Table 7:Prevalence of laboratory derangements of study participants during hospitalization

Variable	Severity	Frequency	Percent (%)
Thrombocytopenia*(n=596)*	Normal	155	26.7
	Mild	154	34.9
	Moderate	162	36.7
	Severe	111	25.2
	Very severe	14	3.2
Hemoconcentration*(n=596)*	By < 10%	200	33.3
	By > 10-14%	284	47.3
	By >15-19%	41	6.8
	By >20%	76	12.6
Leucopenia*(n=596)*	Normal	230	39.1
	Mild (3.0-3.9)	105	28.7
	Moderate (2.0-2.9)	146	39.9
	Severe (1.0-1.9)	111	30.3
	Very severe (<1.0)	4	1.1
AST*(n=573)*	Normal	326	57.1
	Mild (41-200)	184	74.5
	Moderate (201-999)	57	23.1
	Severe (1000+)	6	2.0
ALT*(n=573)*	Normal	396	68.9
	Mild (41-200)	142	79.8
	Moderate (201-999)	32	17.9
	Severe (1000+)	4	2.2

4.7 Complications

Overall, 66 (10.9%) patients out of 601 presented with severe dengue, and among them, four died. The presentation of severe dengue was as follows: end-organ targets in 78.8%, dengue shock in 9.1%, both severe organ target and dengue shock in 6.1%, and severe hemorrhage in 6.1%. The four patients who died presented with multiple organ failure and severe hemorrhage. Among the end organ targets, AKI was observed in 37 patients, followed by myocarditis in 11, ARDS in 8, altered mentation in 8, and severe hepatitis in 6. The average mean length of hospital stay was 4.1 days, with a range of 3.0-5.0 days. This is illustrated in table 8 below.

Table 8:Dengue-related complications during hospitalization

Complications	N	Percent (%)
AKI ***(n=585)***	37	6.3
Dengue myocarditis ***(n=372)***	11	2.9
Dengue shock ***(n=601)***	10	1.7
CNS dengue ***(n=559)***	8	1.4
ARDs ***(n=545)***	8	1.4
Severe hepatitis ***(n=573)***	6	1.0
Severe Hemorrhage ***(n=566)***	4	0.7
Fatal cases ***(n=601)***	4	0.6

4.8 Treatment Modalities

Of the 601 files analyzed, intravenous fluid was administered in 511 (85.0%) of the patients, while 346 (57.6%) patients received antibiotics. 30 (4.9%) patients were transfused, of whom a majority at (80.0%) were given platelet concentrates (Table 9). Five patients were admitted at intensive care unit for ionotropic support and ventilation.

Table 9:Treatment modalities during hospitalization

Variables		Frequency***(n=601)***	Percent (%)
IV fluids		511	85.0
Antibiotics		346	57.6
Blood transfusion	Blood products	32	5.0
	Platelet concentrates	24	80.0
	PRBCs	4	12.5
	Whole blood	4	12.5
Critical care			
	Ionotropic support	4	0.7
	Ventilation	5	0.8
	Dialysis	2	0.3

5.0 CHAPTER FIVE: DISCUSSION

Dengue poses a diagnostic challenge, partly due to its atypical presentation and overlap of symptoms with other febrile illness, especially in endemic regions with limitation of rapid diagnostic kits (10) (26). Prolonged hospitalization, cost of care, morbidities and mortalities related to dengue has imposed substantial economic, social, and personal burden. This retrospective study describes the clinical and laboratory characteristics, treatment modalities, disease severity and outcomes among patients admitted due to dengue infection. The evidence generated is crucial for early diagnosis, improving patient triage and prompt management.

In our study, the median age was 34 years, where more than 65% of the study participants were between 13-40 years of age, with a male predominance pattern, in agreement with other studies (34)(35)(46).

Secondary dengue was documented in 12.7% of patients in our study, in agreement with other African studies(34). An earlier study in Kenya reported a slightly lower prevalence of secondary infection at 9.7%, thus, future concerns of subsequent increase in severe cases of dengue. The viral serotype that produces secondary infection and in particular the pattern of serotype sequence during subsequent infection is crucial to ascertain the severity of the disease. Although all the four dengue serotypes are able to produce severe disease, studies in Thailand have revealed that the DENV-1/DENV-2 sequence of infection was associated with a 500-fold risk of severe dengue compared with primary infection (86).

Our clinical presentation data are similar to those reported in a series of other studies; most of our patients presented with fever, headaches, myalgia, arthralgia and retro-orbital pains (33)(34)(46).

Warning signs that included minor bleeding, clinical fluid accumulation, hepatomegaly, rising hematocrit, and severe thrombocytopenia, were demonstrated in 26.9% of patients. Similar findings were observed in other studies, including in Dar salaam (34). To assist clinicians in early detection of severe disease progression, the 2009 WHO dengue guidelines recommended the use of warning signs as early indicators of plasma leakage (26)(27). Early recognition of warning signs enables effective triaging and prompt intervention especially among suspected high-risk patients, hence reducing morbidity and hospital stay.

In our study, mild muco-cutaneous bleeding was observed in 10.9% of the study participants, with a predominant mucosal bleeding. Similar findings were reported in other studies, including Tanzanian study (34). However, two Cuban studies documented a predominant cutaneous bleeding (58)(59). The variations in the site and severity of bleeding among patients

with dengue could be explained by differences in both viral and host factors (58)(59). Bleeding as a clinical entity ranges widely from minor skin hemorrhage to life threatening GIT bleeding (27). Early detection of bleeding in the febrile phase could be a prognostic sign for progression to severe disease and has been frequently associated with poor outcomes including end organ targets and prolonged hospitalization (26)(27)(91).

Clinical fluid accumulation as a warning sign, was confirmed in 2.8% of patients in our study. Similarly, lower prevalence of clinical fluid accumulation as manifested either by pleural effusion or ascites was reported at 0.9% in Tanzanian study (34). However, higher frequencies of ascites and pleural effusion have been reported in a series of other studies worldwide (61). In a study conducted in Thailand, daily ultrasound examinations of the abdomen and right thorax were performed using a portable ultrasound scanner among the 158 study participants; a higher prevalence of pleural effusion and ascites at 62% and 52% respectively was confirmed (61). Thus, variations and under-reporting of clinical accumulation might be attributed to limitation in utilizing routine ultrasonography as a screening tool of plasma leakage especially in low to middle income countries. The evidence of clinical fluid accumulation early during the febrile phase could be a clinical marker of severe plasma leakage, and further be used as an early predictor of progression to severe disease (26).

Thrombocytopenia was the commonest hematological derangement observed in our study. Approximately 73% of the patients had low platelet count during hospitalization, in agreement with many studies worldwide (35)(46). However, a study in Tanzania reported a lower prevalence of thrombocytopenia at 16% among patient with dengue (34). A single and higher threshold of defining thrombocytopenia and could explain the findings observed in Dare salaam study (34). Thrombocytopenia in dengue widely varies, however, a majority of the average platelet counts in non-severe dengue groups are higher than 100 000 cells per dL. Although low platelet count has been significantly associated with disease progression, and confirmed as one of the key warning signs, no robust clinical evidence of a platelet count cutoff to determine the risk of SD has been identified. A cutoff of 100 000 cells per dL has been however recommended from the 1997 WHO dengue guidelines and 2011 SEARO dengue guidelines (26)(27).

According to 2011 SEARO dengue guidelines, hematocrit change of 10-15% from the baseline is common among patients with dengue infection. This is in agreement with our review. A change of hematocrit by more than 20% as a warning sign, has been however associated with significant plasma leakage and progression of disease severity especially concurrent with moderate to severe thrombocytopenia in the critical phase (44). In our study,

hemoconcentration as manifested by a change of hematocrit by more than 20% was documented in 12% of the patients, in agreement with other African studies (46). Lower prevalence of hemoconcentration demonstrated in our study compared with other studies worldwide might be attributed to the use of larger volumes of intravenous fluids during admission in attempt of ensuring adequate volume replacement.

Progressive leucopenia with a predominant neutrophil count occurs early during the febrile phase. This is followed by neutropenia and relative lymphocytosis characterized by a neutrophil to lymphocyte ration of less than 1 (26)(27). Low white cell count and a positive tourniquet test have been shown to have a positive predictive value of more than 80% in clinical diagnosis of dengue (11)(30). However, severity of leucopenia has not been associated with disease progression (11)(30). In our review, leucopenia was documented in more than 60% of cases, in agreement with most studies worldwide. However, similar studies conducted in Tanzania and Ethiopia demonstrated lower frequencies of leucopenia at 33% and 29% respectively (34)(46). These variations could be attributed to their measuring of cell counts only at admission and using of higher threshold of leucopenia (34)(46).

Hepatic manifestations may range from asymptomatic transaminitis to acute fulminant liver failure (70)(71).Mild to moderate elevation of the aminotransferases has been reported in several studies, with a rise of AST more prominent than ALT, suggestive of non-liver sources of AST (44)(36). In our study, AST and ALT were elevated in 43.1% and 30.8% of the study participants, respectively, in agreement with a series of other studies. In a study conducted in Ethiopia, the elevation of AST and ALT levels among patients with dengue were recorded in 45% and 17.5% respectively (46). However, higher frequency of transaminitis has been reported in other studies worldwide. Variations in the prevalence and severity of transaminitis could be attributed to differences in the viral genotype and serotype, host clinical response, or other host factors including co-infection with chronic hepatitis viruses. The 2009 WHO dengue guidelines did not include transaminitis as one of the warning signs. However, in a meta-analysis study conducted recently, concentrations of AST or ALT higher than three times the upper limit of normal were significantly associated with progression of disease severity. These findings support their monitoring during the febrile phase of the illness.

In our study, the frequency of patients who had severe dengue was 66 (10.9%). This is significantly lower compared to findings in other studies worldwide (90)(91). Although viral factors plays a role in determining disease severity, host factors including African ancestry background characterized by protective genetic and environmental factors, could be attributed to protection against severe dengue in our continent (34)(87)(88). However, our review

demonstrated higher prevalence of severe dengue compared to a study conducted in Dare Salam, where only 4.7% of patients had severe disease (34). This could be attributed to the fact that severe organ targets as part of disease severity spectrum was not captured in the analysis (34). SD has been associated with poor outcomes including prolong hospital stay and mortalities.

Among the dengue-related complications, acute kidney injury was documented in 6.3% of patients. In contrast to a study conducted in Ethiopia, raised creatinine was reported in up-to 19.6% of dengue cases (46). A single interpretation of creatinine at admission without a baseline value could explain the higher frequency of AKI in the Ethiopian study (46). Acute kidney injury among patients with dengue may range from 0.9% to 70% (79)(80). Although both viral and host factors majorly determine the occurrence of severe organ targets, other factors including differences in AKI classification as applied in different studies, might also explain the wide variations in the prevalence of DAKI.

Incidence rates of neurological complications as atypical symptom of dengue infection vary from 0.5%-20% in recent years. In our analysis, only 1.4% of the study patients had evidence of CNS dengue. Similarly, lower incidence of neurological signs were reported in a Tanzanian study (34). This could be attributed to underdiagnosing and underreporting of dengue-related neurological complications. Although most patients with CNS dengue present with altered mentation or seizures, a few may not present with any neurological symptoms (69). In such cases, the diagnosis of CNS dengue may be supported by virus detection in culture or PCR and serological analysis of cerebrospinal fluid as confirmed (68).

Dengue-related cardiomyopathy can vary from asymptomatic non-specific ECG abnormalities through to a more severe clinical manifestation (72)(73)(74)(75) . The transient asymptomatic nature of the clinical entity increases risk of underdiagnosis and under-reporting of dengue myocarditis (72)(73)(74)(75). In our study, dengue myocarditis was documented in 2.9% of the cases as confirmed by ECG, ECHO or cardiac enzymes, especially among patients with underlying cardiovascular diseases. Although dengue-related myocarditis has not yet been described in any other African countries, worldwide, ECG abnormalities were observed in 30-40% of dengue cases, and are usually transient and non-specific (75)(76). Additionally, elevated troponins and brain natriuretic peptide was demonstrated in 15% of the dengue patients, while cardiomyopathies with resultant systolic and diastolic dysfunction have also been reported in a series of other studies (74)(75) (77). Thus, routine non-invasive clinical monitoring with electrocardiogram, echocardiogram or cardiac biomarkers in all high-risk patients could explore the true burden of dengue-related cardiomyopathy in Africa.

Prolonged hospital stay has not only been associated with severe dengue, but also in patients with non-severe infection (84)(85). In our study, the average duration of hospital stay was 4.1 days. Early diagnosis, improving patient triage, and prompt management of suspected patients with dengue may improve health and economic impact of the infection in terms of hospital stay (26)(27).

For a disease that is complex in its manifestations, management is relatively inexpensive and effective so long as early recognition and timely intervention are instituted to achieve a good clinical outcome. Admission and prompt management of suspected cases with warning signs, severe plasma leakage or severe organ targets is crucial. Otherwise, for patients with classical dengue without warning signs, symptomatic treatment to control pyrexia and analgesia is sufficient (26)(27).

In our analysis, out of 601 cases, a majority (68%) were admitted with dengue fever without warning signs. However, more than 85% of all the patients analyzed were given intravenous fluid therapy, despite lower frequency of warning signs and SD. This is in contrast to 2009 WHO dengue guidelines, which has clearly emphasized on the role of intravenous fluids to only those with warning signs and severe dengue (26)(27). This disparity could be partly due to inability of the patients to feed orally. In such patients, isotonic solutions including crystalloids is generally recommended to replace the plasma losses, however, in the case of hypotensive shock, colloids solutions is preferred (26)(27).

The 2009 WHO dengue guidelines recommends the use of fresh packed red cells or whole blood in patients with severe overt bleeding if blood transfusion is considered (26)(27). Routine prophylactics platelet transfusion for severe thrombocytopenia has not been shown to be effective and is not necessary (26)(27). The use of blood components such as platelet concentrates, FFP or cryoprecipitate could contribute to fluid overload. Although the 2011 WHO SEARO dengue guidelines generally does not recommend prophylactic platelet transfusion, it may be considered in adults with underlying hypertension and very severe thrombocytopenia of less than 10,000 cells per dL. In our study, 32 (5.0%) patients were transfused, with a majority of them (75.0%) receiving platelets concentrates due to thrombocytopenia of less than 30,000 cells per dL in the absence of documented bleeding.

Hemodynamic response and serial hematocrit counts are essential to avoid fluid overload with resultant ARDS while ensuring adequate volume replacement. Vasopressors and ionotropic therapies as temporary measures to prevent life-threatening hypotension in dengue shock may be considered in selected patients (26)(27).

In our study, approximately 57% of patients received antibiotics. Although all the study participants were admitted due to laboratory-confirmed dengue infection, antibiotics were administered in more than half of the cases during their hospital stay. The 2009 WHO dengue guidelines encourages clinicians awareness and monitoring of concurrent bacteremia and bacterial coinfection among dengue patients, however, limited data is available (26)(27). In a recent audit summarizing clinical studies and case reports, 0.18-7% of dengue infection were accompanied by concurrent bacteremia, while 14.3-44.4% of dengue related deaths seem associated to bacterial coinfections. Co-morbidities, advance age and more SD manifestations could be risk factors for dual infections. Despite the real burden and consequences of this emerging concern is still not computable accurately, greater awareness by the clinicians is warranted to early predict the risk of concurrent bacteremia and bacterial coinfection. Additionally, the inappropriate use of antibiotics has detrimental effects on patients, health care system, and society especially in the settings where antibiotics stewardship policy has not been well implemented yet.

Supportive care and adjuvant therapy including renal replacement therapy for DAKI, cardiac intervention for conduction abnormalities, and ventilation may be warranted in selected patients with SD. In our study, 5 patients were admitted at intensive care unit for ionotropic support and ventilation (11)(30)

6.0 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The study describes the clinical and laboratory characteristics, treatment modalities, disease severity and complications among patients admitted due to dengue infection in the coastal city of Mombasa-Kenya.

The findings clearly shows that most of our patients admitted due to dengue infection had classical non-severe disease. However, distinct clinical, hematological, biochemical, and complications related to the infection were also demonstrated, especially among individuals with warning signs (DFWS) and severe dengue. Higher than expected cases of warning signs and end organ targets were documented, with a majority of the patients hospitalized more than 3 days.

The main limitation of the study was missing information, especially of data on severe organ targets. To mitigate this, the principal investigator collected data himself, ensuring that every piece of information from triage and clerking notes, laboratory and imaging reports, and treatment sheets was captured.

Our study was one of the biggest studies in Africa which extensively assessed dengue-related complications. The retrospective survey allowed a large number of unselected dengue cases to be analyzed, giving power to the conclusions drawn from the study.

Public awareness, routine use of diagnostic kits coupled with vigilant monitoring by clinicians will enable early recognition and prompt treatment, hence, good clinical outcome.

Routine performance of relevant hematological, biochemical and imaging investigations is recommended to describe the true incidences of end-organ targets. Future prospective studies are needed to ascertain the predictors of severe disease and its associated morbidities. Improved record keeping will aid in future data collection. Establishment of national dengue control authorities facilitate, appropriate health infrastructure, effective vector control strategies, and allocation of financial budget are required for future anticipated outbreaks.

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APPENDICES

Appendix I: Study Proforma

Date…… **Study number……**

Age……..

Date of Admission……..

Date of Discharge…….……

Duration of Hospital Stay……..….

A: Demographics

NO	**Question**	**Response**	**Code**
1	Gender [GEN]	F = 1 M= 2	**[ ]**
2	Age [A]	Number	
3	Residence		
4	Occupation	Self-employed 1 Employed 2 Unemployed 3 Retired 4	**[ ]**
5	Education	None 1 Primary school 2 Secondary school 3 Tertiary school 4	**[ ]**
B: Co-Morbidities			
6	Hypertension	Yes 1 No 2 Not documented 3	**[ ]**
7	Diabetes	Yes 1 No 2 Not documented 3	**[ ]**
8	Others	Yes 1 No 2 Not documented 3 If YES, indicate specific……	**[ ]**
D: Type of Diagnosis			

9	Primary dengue	Yes 1 No 2 Not documented 3	**[ ]**
10	Secondary dengue	Yes 1 No 2 Not documented 3	**[ ]**
C: Clinical Presentations			
9	Fever	Yes 1 No 2 Not documented 3	**[ ]**
10	Headache	Yes 1 No 2 Not documented 3	**[ ]**
11	Retro-orbital pains	Yes 1 No 2 Not documented 3	**[ ]**
12	Myalgia/Arthralgia	Yes 1 No 2 Not documented 3	**[ ]**
13	Hematemesis	Yes 1 No 2 Not documented 3	**[ ]**
14	Melena	Yes 1 No 2 Not documented 3	**[ ]**
15	Hematochezia	Yes 1 No 2 Not documented 3	**[ ]**
16	Gum bleeding	Yes 1 No 2 Not documented 3	
17	Epistaxis	Yes 1 No 2 Not documented 3	
18	Others	Yes 1 No 2 Not documented 3	
D: Vitals			
16	Tachycardia	Yes 1 No 2 Not documented 3	**[ ]**
17	Systolic hypotension	Yes 1 No 2 Not documented 3	**[ ]**
18	Narrow pulse pressures	Yes 1 No 2	**[ ]**

		Not documented 3	
E. Dermatological			
19	Rash	Yes 1 No 2 Not documented 3	**[ ]**
20	Petechiae	Yes 1 No 2 Not documented 3	**[ ]**
21	Ecchymoses	Yes 1 No 2 Not documented 3	**[ ]**
F: Neurological			
22	Altered mentation	Yes 1 No 2 Not documented 3	**[ ]**
23	Seizures	Yes 1 No 2 Not documented 3	**[ ]**
G: Respiratory			
24	Pleural effusion	Yes 1 No 2 Not documented 3	**[ ]**
25	ARDs	Yes 1 No 2 Not documented 3	**[ ]**
H: GIT System			
26	Liver enlargement>2cm	Yes 1 No 2 Not documented 3	**[ ]**
27	Ascites	Yes 1 No 2 Not documented 3	**[ ]**
I: Cardiovascular			
	Myocarditis	Yes 1 No 2 Not documented 3	**[ ]**
	Severe hemorrhage	Yes 1 No 2 Not documented 3	**[ ]**

J: Laboratory			
28	Hb level	At admission = 24-72 hours= Nadir= Peak=	
29	HCT	At admission = 24-72 hours= Nadir= Peak=	
30	Platelets	At admission = 24-72 hours= Nadir= Peak=	
31	WBC count	At admission = 24-72 hours= Nadir= Peak=	
32	Lymphocytes	At admission = 24-72 hours= Nadir= Peak=	
33	Monocytes	At admission = 24-72 hours= Nadir= Peak=	
34	Neutrophils	At admission = 24-72 hours= Nadir= Peak=	
35	Creatinine levels	At admission = 24-72 hours= Nadir= Peak=	
36	AST	At admission = 24-72 hours= Nadir= Peak=	

37	ALT	At admission = 24-72 hours= Nadir= Peak=	
K: Treatment modality			
	Intravenous fluids	Yes 1 No 2 Not documented 3	**[ ]**
	Antibiotics	Yes 1 No 2 Not documented 3	**[ ]**
	Platelet transfusion	Yes 1 No 2 Not documented 3	**[ ]**
	Packed RBC	Yes 1 No 2 Not documented 3	**[ ]**
	Whole blood	Yes 1 No 2 Not documented 3	**[ ]**
	Ventilation	Yes 1 No 2 Not documented 3	**[ ]**
	Ionotropic support	Yes 1 No 2 Not documented 3	**[ ]**
	Dialysis	Yes 1 No 2 Not documented 3	**[ ]**
K: Survival status			
38	Alive and discharge	Yes 1 No 2 Not documented 3	**[ ]**
39	Fatal case	Yes 1 No 2 Not documented 3	**[ ]**