A Current Review on the Pathophysiology and Diagnostic Aspects of Dengue

By

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Bachelor of Pharmacy

School of Pharmacy
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April, 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “A Current Review on the Pathophysiology & Diagnostic Aspects of Dengue” submitted by Tahmina Akhter Habiba (18346073) of Summer, 2018 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on April, 2023.

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Ethics Statement

The review has been conducted in accordance with the ethical guidelines and procedures. In addition, the author has also ensured that the information presented in this review is accurate and based on the most current and reliable sources. Lastly, have provided appropriate citations for all sources of information and have avoided any plagiarism or misrepresentation of the work of others.

Abstract

Dengue fever is a viral disease transmitted by mosquitoes, affecting a significant number of people worldwide. This review aims to summarize the current knowledge of the pathophysiology and diagnostic aspects of dengue fever. The Pathophysiology of dengue is well understood so far; which eventually aims to explain the association of inflammatory response followed by the release of cytokines and chemokines which later allied with causing fever, rash, headaches etc. T-cells and the gut microbiome role in worsening the condition during dengue are also highlighted. Additionally, this review explores clinical manifestations of dengue fever and severe dengue-associated complications. Molecular, virological, and serological assays are explored as diagnostic methods, with challenges associated by their use. New diagnostic technologies, including (SPR), (QCM), (EIS), (SERS) and CRISPR-Cas assays are discussed, with SPR being the most effective due to its high sensitivity and label-free method. Overall, the review highlights the importance of accurate and timely diagnosis in managing dengue fever.

Keywords: Dengue, Transmission, Pathophysiology, Dengue-associated-complications, Diagnostic tools, clinical stage, Advanced technologies, Treatment

Dedication

This review paper is dedicated to my parents and brother for their immense support and hope, as well as to my supervisor for her valuable guidance.

Acknowledgement

I acknowledge my gratitude to Professor Dr. Eva Rahman Kabir, Dean, School of Pharmacy, BRAC University, for allowing me to complete my undergraduate review paper. I am much indebted to Professor Dr. Hasina Yasmin, Programme Director and Assistant Dean, School of Pharmacy, BRAC University.

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List of Acronyms

DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
DENV	Dengue Virus
NS1 protein	Non-structural protein 1
AKI	Acute kidney injury
ADE	Antibody-dependent enhancement
EDS	Expanded dengue syndrome
TTP	Thrombotic thrombocytopenic purpura
IgM	Immunoglobulin M
ELISA	Enzyme-linked Immunoassay
IgG	Immunoglobulin G
PCR	Polymerase chain Reaction
POC	Point-of-care
QCM	Quartz Crystal Microbalance
SPR	Surface Plasmon Resonance
EIS	Electrochemical Impedance Spectroscopy
SERS	Surface-enhanced Raman spectroscopy
ssDNA	Single-stranded DNA viruses
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
Ag	Antigen
CRISPR	Clustered regularly interspaced short palindromic repeats
IV	Intravenous

Chapter 1: Introduction

1.1 Background

The infectious agent that causes dengue fever, also known as break-bone disease, is a mosquito-borne tropical disease also known as break-bone disease (Nisha et al., 2020). Whereas, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) have all seen a significant rise in prevalence throughout the world during the last three decades, coinciding with an accompanying rise in the occurrence of outbreaks (Asidik et al., 2021). There are an estimated 2.5 billion individuals who reside in regions that are endemic to dengue fever and are exposed to its vector and are at risk of contracting the disease. Annually, 105,000 people are affected by the dengue disease (Bignardi et al., n.d.). The spread of dengue fever across the world may be attributed to a number of different causes, such as population increase, growth in population, uncontrolled fast urbanization and building, ecological degradation, the lack of dependable piped water, and inefficient vector control techniques (Kayesh et al., 2023).

DENV is a member of the Flaviviridae family of viruses. It's an enclosed virus with a single strand of positive-sense RNA (Troost & Smit, 2020). It contains a total of four distinct serotypes, which are designated as DENV-1, DENV-2, DENV-3, and DENV-4 (Troost & Smit, 2020) (Guzman et al., 2016). During an epidemic that took place in 2007, it was recently hypothesized that a fifth serotype of DENV isolated in Malaysia emerged (Bignardi et al., n.d.). Mosquitoes belonging to the genus Aedes, namely the *Aedes aegypti* and *Aedes albopictus* species, are responsible for the vast majority of instances of dengue fever that occur across the globe (Nisha et al., 2020).

In terms of how the disease shows up in a person's body, Bhat et al. (2015) say that a person with dengue often has fever and a headache. Infection with one of the four serotypes of the dengue virus, which are highly correlated with one another, may induce a variety of symptoms, including headache, fever, nausea, vomiting, rashes, myalgia, and arthralgia (Khan et al., 2022). There is currently no specific cure for dengue; however, people who have the more severe form of the disease, dengue hemorrhagic fever, may sometimes be saved by timely medical attention (Asidik et al., 2021).

Dengue virus disease manifestation can occur in one of two ways: symptomatic or asymptomatic. Dengue's clinical presentation varies by virus strain and host characteristics, including age, immunological state, etc., and may be divided into three separate categories: dengue fever (DF), undifferentiated febrile sickness (viral syndrome), and dengue hemorrhagic fever (DHF) with dengue shock syndrome (DSS) (Asidik et al., 2021).

Table 1: Classification of Dengue based on their disease manifestation with severity(Asidik et al., 2021)

DF/DHF	Grade	Symptoms	Laboratory
DF*		Fever with two or more of the following signs: headache, retro-orbital pain, myalgia, arthralgia plus positive tourniquet test	Leucopenia occasionally Thrombocytopenia, may be present, no evidence of plasma loss
DHF	I	Above signs plus positive tourniquet test	Thrombocytopenia ≤ 100,000/ µL, hematocrit rise ≥ 20%
DHF	II	Above signs plus spontaneous bleeding	Thrombocytopenia ≤ 100,000/ µL, hematocrit rise ≥ 20%
DHF	III**	Above signs plus circulatory failure (weak pulse, hypotension, restlessness)	Thrombocytopenia ≤ 100,000/ µL, hematocrit rise ≥ 20%
DHF	IV**	Profound shock with undetectable blood pressure and pulse	Thrombocytopenia ≤100,000/ µL, hematocrit rise ≥ 20%

DF: dengue fever; DHF: dengue hemorrhagic fever; *Modified WHO criteria; **DHF Grade III and IV are also called as dengue shock syndrome (DSS).

1.2 Epidemiology of Dengue

In the tropics and subtropics, mosquito-transmitted dengue fever poses a serious threat to public health (Lee et al., 2022). Over forty percent of the population is now at risk of catching the Dengue virus, mostly in tropical and subtropical zones (Abdulsalam et al., 2022).

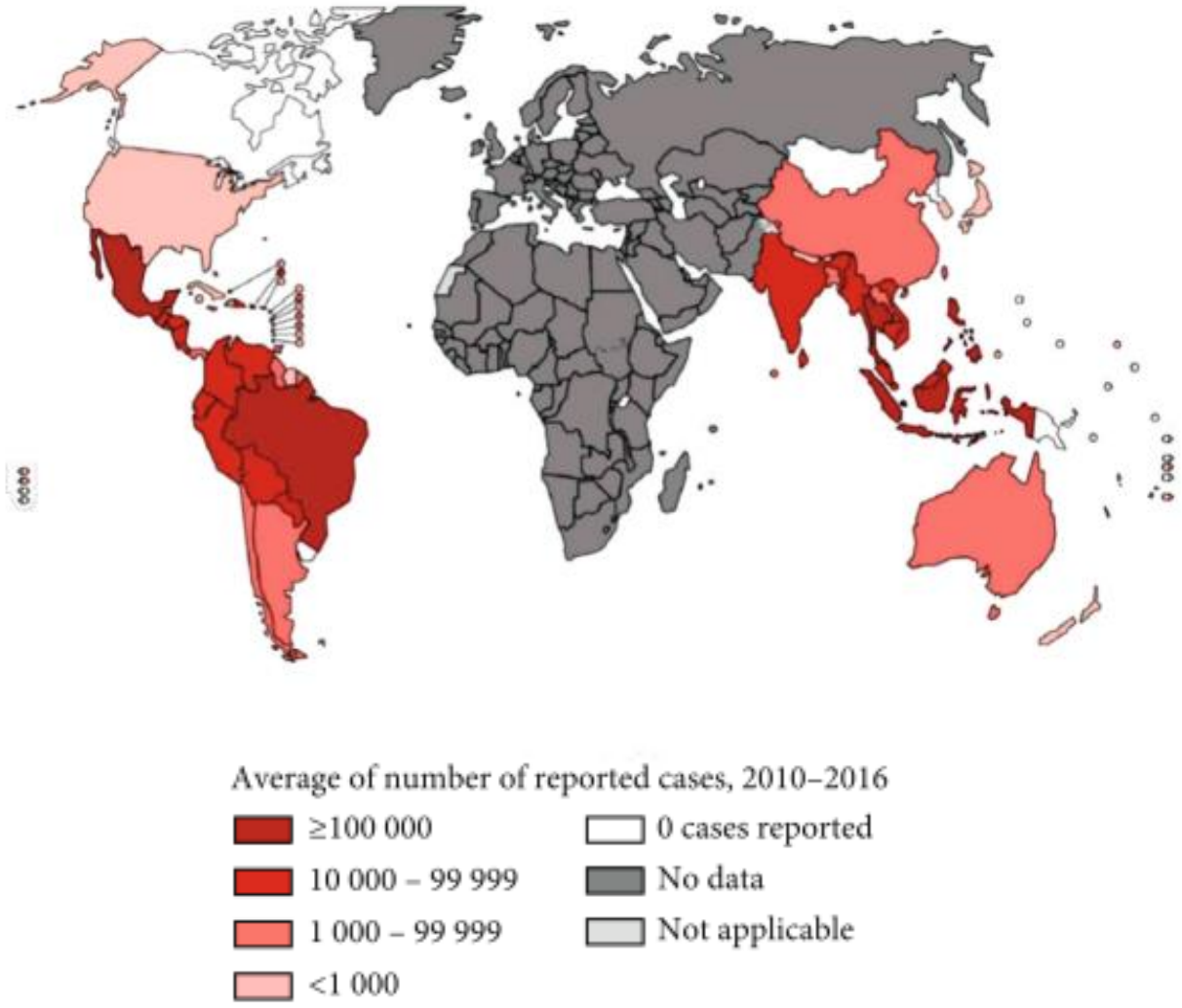

Figure 1: Global distribution of Dengue by geographical locations and average number of reported cases for the time period of 2010–2016 (Islam et al., 2021); here, dark red areas have the highest transmission rate and reported cases, which come down to narrower numbers in the grey and white zones.

In addition, dengue fever is occurring as an endemic disease in hot and warm areas, and its frequency is growing in southern Asia, the western Pacific, the Americas, and Africa. At the moment, there are more than one hundred nations in the world that are affected by dengue fever (Sugianto, 2021). Over eighty percent of the reported 100–400 million people affected every year are either quite mild or show no symptoms at all (Trivedi & Chakravarty, 2022). Since the first known epidemic in 1779 in Jakarta, Indonesia, DHF has been a public health problem linked with significant morbidity and death (Sugianto, 2021). WHO also pointed out that the number of dengue incidences has increased by an additional eight times over the past 20 years, from 505,430 in 2000 to more than 24000 in 2010 and 52000 in 2019 (Trivedi & Chakravarty, 2022).

The global expansion of dengue fever may be attributed to a variety of factors, including a growing human population, an increase in urbanization and development without sufficient planning, the impacts of climate change, a lack of access to piped water, and ineffective vector control efforts (Kayesh et al., 2023) (Guzman et al., 2016). There is a possibility that the fast expansion of dengue viruses into variants that are more infectious is possibly adding to the recent emergence of the sickness (Trivedi & Chakravarty, 2022). The fast global spread of dengue is attributed in part to the increasing popularity of air travel, according to researchers. At 75% each, the Western Pacific and Southeast Asia carry the lion's share of the global illness burden. DENV-3 is currently the main strain in Asian nations, having superseded DENV-2 as the major serotype (Trivedi & Chakravarty, 2022). Officials in Bangladesh documented the world's first dengue pandemic in 2000, which resulted in 5551 cases and 93 deaths (Kayesh et al., 2023). The prevalence of dengue is substantially greater in the top three transient locations, which are Dhaka, Chittagong, and Khulna, compared to the more scattered remote rural areas in the remainder of the nation's 50% urban population (Paul et al., 2021).

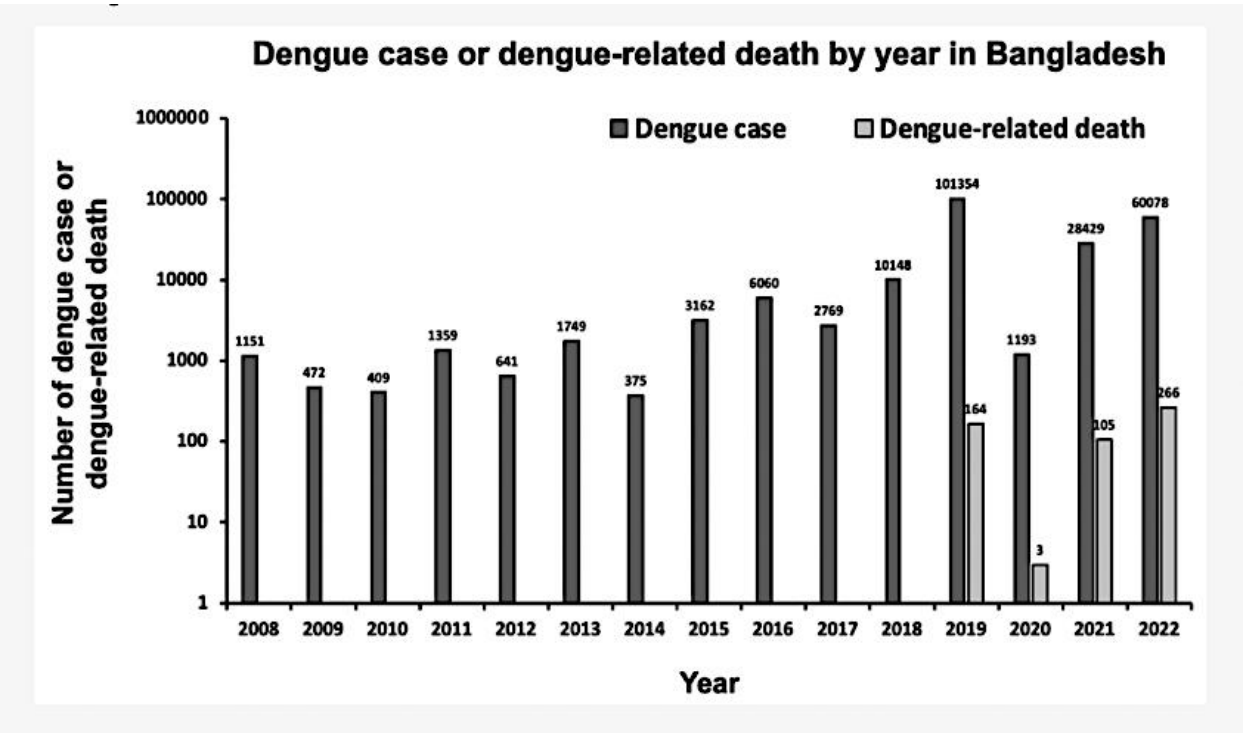

Figure 2: Number of reported dengue cases and dengue-related deaths per year in Bangladesh between 2008 and 10 December 2022 (Kayesh et al., 2023) Here the black lines are dengue cases and the slightly grey lines are the number of deaths associated with dengue; it's visible that the number of dengue cases has dramatically risen over this time period, with the maximum cases and reported deaths at 2019; but a surprising fact is that until 2022 the number of deaths have risen well in comparison to the associated dengue cases.

1.3 Etiology of Dengue Fever

Affected by any one of these four serotypes (DENV-1, -2, -3, and -4) of the dengue virus, which can pose infection and spread from person to person, is considered an endemic disease. The infection confers protection against future infections of the same subtype throughout a person's life, but not against infections caused by different serotypes (Trivedi & Chakravarty, 2022). When compared to initial infections, secondary or subsequent infections have a much higher likelihood of being linked with serious clinical symptoms (Jaenisch et al., 2016) (Chuansumrit & Tangnarara, 2006).

When a mosquito with the dengue virus bites a person, the virus first gets into the body through the skin. Afterward, the majority of the viral replication takes place inside the macrophages (Hasan et al., 2016). Macrophages will quickly respond by grabbing the virus and digesting it so they can change into APC, which stands for antigen-presenting cells (Sugianto, 2021).Because of the antigen that is associated with this macrophage, T-helper cells will be activated, and other macrophages will be drawn in to phagocytes more viruses. When infected macrophages meet a T helper cell, T cytotoxic cells will kill them because they have been turned on. The process that results in the secretion of mediators causes fever, joint discomfort, muscular soreness, malaise, and other systemic symptoms (Sugianto, 2021).In addition to this, infection may also be caused by a direct infection of the skin caused by the virus, as well as by immunological and chemically mediated mechanisms that are generated by the contact between the host and the virus (Hasan et al., 2016).

As was said above, secondary infections can cause unusual immune responses, such as the release of cytokines and chemokines, the activation of T lymphocytes, and the disruption of the body's hemostatic system (Trivedi & Chakravarty, 2022) (Chuansumrit & Tangnarara, 2006). Innate, cellular, and humoral reactions of the host are all thought to have a role in the course of the disease; however, the manifestation of additional unembellished experimental indications occurs only after the virus has been eradicated from the host body (Hasan et al., 2016). When the virus first enters the body, clinical manifestations of DF will begin to appear. The virus will replicate in the circulation, where macrophages will eventually engulf it for digestion. Later, viremia starts two days before symptoms appear and lasts for five days after the fever starts (Sugianto, 2021).

1.4 Transmission of Dengue

Individually, any of the four types of dengue virus may be spread through the bite of a diseased Aedes mosquito (Sugianto, 2021) (Vega et al., 2016). Despite the fact that *Aedes aegypti* is the main reason, other mosquito species are responsible for nearly two-thirds of the world's dengue reports. As a result, it is evident that additional transmission of dengue virus by nearby mosquitoes is a possibility whenever the virus is transported to a new environment (Wiwanitkit, 2010). The female *Aedes aegypti* mosquito bites humans to get protein for her eggs and inoculates the dengue virus during the initial febrile and viraemic phases of disease, which are responsible for transmission (Fernandez Cerna et al., 200 C.E.).

During the stage of external incubation, the virus gets into the mosquito's midgut and infects the cells there. It then spreads throughout various tissues within the insect and proceeds to replicate. Finally, after a period of 5–12 days, usually around 8–10 days, the virus infects the mosquito's salivary glands. After the salivary glands of the mosquito have been affected, the mosquito is infectious and is capable of passing the virus on to another person while it is feeding on their blood. The mosquito is infectious for its whole lifespan and has the potential to spread the disease to anybody it later bites or probes while attempting to feed on (Guzman et al., 2016). On the other hand, the viral infection that occurs in humans begins to build up significant titers two days prior to the commencement of the fever (when the patient is non-febrile) and continues for five to seven days after the fever has begun (febrile). Only at these two times of the year can the mosquito species get infected with the disease. After then, human beings become the source for transmission (Asidik et al., 2021).

According to the WHO, biotic and abiotic variables both have a role in the spread of the dengue virus. Temperature, humidity, and precipitation are examples of abiotic variables. Vector control may be beneficial in reducing dengue transmission; however, many dengue-

endemic regions do not have efficient vector monitoring and control programmes (Chan et al., 2013).

Table 2: Transmission of Dengue virus according to WHO(Asidik et al., 2021)

Transmission Cycle	**Characteristic features**
Enzootic cycle	• Primitive sylvatic cycle • Monkey-Aedes-monkey cycle • Viraemia lasts for 2–3 days • All 4 serotypes isolated from monkey
Epizootic cycle	• Crosses over to non-human primates • Among touqe macaques (Macaca sinica)
Epidemic cycle	• Human-Aedes aegypti-human cycle with • Ae. aegypti is the efficient vector • Disease progresses with high viral titres

1.5 Life Cycle of Dengue Virus

In the past, DENV was mostly spread in Asia and Africa by the Aedes mosquito and nonhuman primates through sylvatic cycles, with humans only showing up rarely. However, in the modern era, the spread of DENV around the globe is a consequence of the virus's development in all forms of transmission (e.g., sylvatic cycles and vertical: mosquito to mosquito) (Islam et al., 2021).

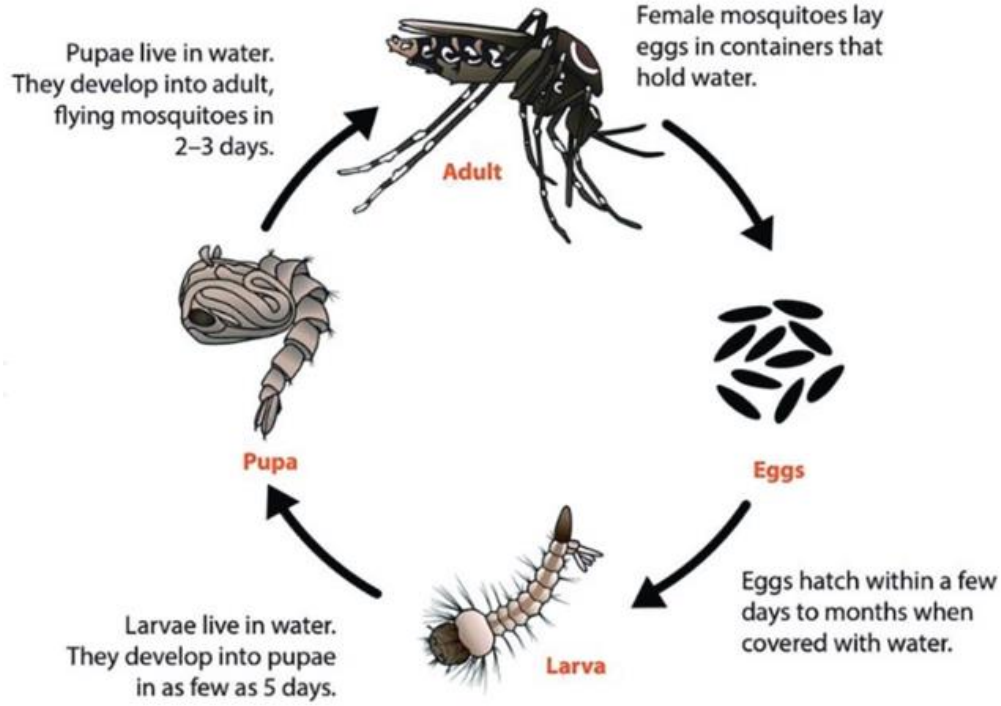

Figure 3: Life stages of *A. aegypti* (Fernandez Cerna et al., 200 C.E.); here egg, larva, and pupa are immature stages and aquatics as they require water to deposit for these stages to get completed; whereas these deposits are called breeding sites; eggs require few days to months, larvae require 5 days, and pupae require 2-3 days to proceed into the next stage and become the adult mosquito.

The mosquito is responsible for the pathogen's transmission to its host. Insects play a crucial role in the transmission of illness by feeding on the blood of infected people and animals (Trivedi & Chakravarty, 2022; Zhu & Cheng, 2023). Therefore, the disease may be readily transmitted by vectors. The Aedes mosquito cannot acquire the disease from an unaffected host, therefore stopping the cycle of reproduction. In the early phases of a virus transmitted by mosquitoes, it is crucial to reduce the number of people affected by this disease. Throughout their regular (30–45-day) life cycle, mosquitoes are capable of transmitting the virus via their trans ovarian route (Trivedi & Chakravarty, 2022). Female mosquitoes are able to spread dengue because they infect their victims with the virus while sucking their blood for nutrition (Fernandez Cerna et al., 200 C.E.).

1.6 Treatment of Dengue Fever

Given the enormous prevalence of dengue-related illnesses, there is currently no widely available vaccine or authorized antiviral therapy to cure or mitigate dengue virus replication (Troost and Smit, 2020; Bhat et al., 2015). Hence, a key pressing requirement in the therapy of dengue is a secure and potent antiviral medication. Despite the fact that certain antiviral aspirants have undergone controlled, randomized trials, the outcomes are therefore unsatisfactory (Guzman et al., 2016). This will include chloroquine, celgosivir, and balapiravir, a polymerase inhibitor created to combat the linked virus known as hepatitis C (a cellular glucosidase inhibitor). Here are some few guidelines and recommendations to consider for the treatment of dengue,

- Hospitalization is recommended for patients with warning symptoms, severe dengue, or in certain conditions such as being young, old, pregnant, diabetic, or residing alone.
- Arterial tube may be necessary for accurate blood pressure measurement, regular blood tests, and central intravenous lines for volume replacement and blood replacement (Chawla, Yadav, and Chawla, 2014).
- Warning signs indicate the need to start intravenous (IV) crystalloids and modify electrolyte administration and should be followed at the point of onset.
- In case of shock, colloids may be administered, especially if the patient has not responded well to crystalloid boluses previously.
- Blood transfusion is essential in situations of significant haemorrhage or indication of bleeding when the patient remains unstable despite sufficient fluids and hematocrit continues to decrease.
- Platelet transfusion is recommended if platelet levels fall below 20,000 cells per microliter, regardless of the severity of the risk of bleeding.

1.7 Prevention of Dengue

Dengue is a potentially endemic virus that is rapidly spreading over the globe. Since 1950, the number of dengue fever cases worldwide has multiplied by a factor of 30 (Khan et al., 2022). Early diagnosis is essential for the efficient care of dengue, which is essential for lowering the fatality rate and bringing the disease's severity under control (Wang et al., 2020).

Intervention through immunization has been attempted; however, after failed attempts to create effective immunization, pharmaceutical companies continue to invest in reliable vaccines without definite results (since curative drugs are unable to inhibit the transmission cycle, thereby reducing the incidence of dengue infection) (Fernandez Cerna et al., 200 C.E.). Several antiviral medicines are being researched for use against dengue virus infections, but a safe, effective, and cost-effective vaccine against all four disease-causing types of dengue virus is still required. Therefore, an individual with dengue fever has a possibility of survival if they get immediate medical care. In order to ease the symptoms of fever, paracetamol and other antipyretics may lower body temperature. For joint discomfort, analgesics and other painkillers may provide pain relief. Patients with DHF or DSS must be hospitalized. Oral rehydration may be used to treat dehydration, and intravenous fluid replacement can be used to treat shock if oral administration is challenging. In the case of serious bleeding or a platelet count below 20,000, a platelet transfusion is advised (Kausar, 2022).

1.8 Objective

The main objective of this study is to summarize the pathophysiological and diagnostic aspects of dengue fever. The specific objectives are:

- Finding co-morbidity issues along with dengue fever
- Analyzing new diagnostic methods to give an exceedingly good progress at the detection and treatment of dengue outbreaks.

Chapter 2: Methodology

This methodology is intended to provide a comprehensive overview of the method that was utilized to carry out a literature review on the current pathophysiology and diagnostic features of dengue fever. This information will be presented in the chapter titled "Methodology." In this section, the research methodology, search strategy, data extraction methodology, and data analysis methodology that were utilized in this study are broken down and clarified.

The design of the research that was done for this study was based on a thorough review of the literature. In the field of health research, this format is often used to give a summary of all the information that is already known about a certain topic. The systematic approach starts with coming up with a research question, then comes up with criteria for which studies to include and which to leave out, then does a thorough search for relevant studies, and ends with an analysis of the quality of the evidence. Have looked in every online database that I could find, including PubMed, Medline, Springer, and the Cochrane Library. The search was limited to articles written in English that were published between the years of January 2014 and January 2023. During the search, keywords like "dengue fever," "pathophysiology," "diagnosis," "clinical features," and "treatment" were used. Also, looked through the reference lists of relevant publications by hand, which led me to find more studies. For this review to include studies, looked into specifically about the pathophysiology and diagnosis of dengue fever in human populations. This was one of the criteria for inclusion. The review did not include any studies that concentrated on animal models or that failed to report on these aspects of the research. In the next chapter, a brief summary of the review's findings will be given. This will be done in a way that is organized by the pathophysiology and diagnosis of dengue fever.

Chapter 3: Pathophysiology

Dengue fever's pathophysiology starts when an infected mosquito bites a person and spreads the virus. The virus looks for immune cells like monocytes, macrophages, and dendritic cells and infects them. Infected immune cells will then make cytokines and chemokines, which cause inflammation by bringing more immune cells to the site of the infection (Guabiraba & Ryffel, 2014).During the inflammatory response, cytokines and chemokines are released. These chemicals cause a fever, headaches, pain in the muscles and joints, and a rash. The rash usually shows up on the boot, arms, and legs, and it looks like small red spots that may join together to form bigger red areas. As the body's immune system keeps working, the virus can move to other organs and tissues. The immune response can also accidentally hurt other organs, like the liver and kidneys, which can lead to other problems.

How bad the disease is depends on how the virus and the immune system of the patient work together. Most people who have it don't have any symptoms, but severe cases, especially in children, can be fatal. Most of the time, people who have the dengue virus don't have any symptoms. The dengue virus causes a spectrum of symptoms, including fever, a viral illness, dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (Schaefer et al., 2022).A more severe form of dengue infection, dengue hemorrhagic fever (DHF), is characterized by plasma leakage and intrinsic coagulopathy. DHF happens when cytokines make the blood vessels more permeable and cause albumin to leak out of the blood vessels. A gelatinous layer called the glycocalyx that lines the endothelium of the blood vessels is also involved in controlling how fluid moves. There are several factors involved in the development of DHF, including secondary dengue infection, the immune system, and viral products such as NS1. Immune processes, including antibody-enhanced viral multiplication

and an increased cytokine response, affect vascular permeability, lowering blood volume, blood pressure, organ perfusion, and organ failure (Sarana, n.d. 2020)

Low platelets, issues with blood clotting, and damaged blood vessels are just a few of the factors that can result in bleeding in DHF patients. Studies in the lab show that the prothrombin time is longer, there are fewer coagulation factors, and there is less fibrinogen. There has also been a strong activation of the complement and a drop in the levels of complement components in the blood. Dengue virus can destroy platelets when it binds to them directly or when it binds to an antibody. Either immune system damage or direct damage to cells by dengue viruses can result in dengue hemorrhagic fever. There is a strong correlation between the number of infected monocytes and the severity of dengue hemorrhagic fever (WHO, 1993).

In addition, Dengue yet results in coagulopathy and a decrease of platelets. Your platelet count will be low if you have dengue fever because the virus causes thrombopoiesis in stem cells to be disguised or killed by antibodies targeting the NS1 protein. Patients with dengue fever who have mucosal discharge should be considered for platelet transfusion. Patients and experts seek platelet transfusions when platelet counts fall below 10,000–20,000/ul. When a condition causes the small pores of the blood arteries to expand, resulting in plasma spilling into the extracellular compartment, haem concentrates are produced. Red blood cell count indicates whether or not a patient has been negatively affected by dengue or is on the verge of entering dengue shock (OAJBS.ID.000415). Severe dengue fever can cause damage to the liver, kidneys, and other organs. The damage can lead to organ failure, which can be fatal if not treated promptly and here at table 3, have listed some of the major complications and their available treatment.

Table 3: Complications associated with dengue fever and worsening the symptoms and causing sever pathophysiological changes in the host; current available treatment for these cases

Complications associated with Dengue fever	Etiology	Treatment
Acute kidney injury (AKI) (Bignardi et al., n.d.)	Hypotension-related shock, viral damage, immune-mediated indirect pathways, and rhabdomyolysis are among hypotheses that might play a role (Bignardi et al., n.d.).	Renal replacement therapy, electrolyte balance maintenance, infusion rates and tonicities optimized for the patient's needs, and finally fluid delivery (ideally crystalloid) are the goals of this treatment.
Anterior uveitis, retinal vasculitis and edema, exudative retinal detachment, optic neuritis, ischemic optic neuropathy, and branch retinal artery blockage are all ophthalmic consequences of dengue feverocclusion(Santé et al., n.d.)	Antibody-dependent enhancement (ADE) antibodies facilitate infection by a heterologous serotype of the virus, which is facilitated in part by the individual's preexisting immune system and the release of large quantities of cytokines (Santé et al., n.d.)	Treatment with corticosteroids
Expanded dengue syndrome (EDS) includes conditions like liver failure, myocarditis, and dengue encephalitis (Kaur et al., 2022).	Diseases that are often associated with dengue and that might display EDS include fulminant hepatic failure, acalculous cholecystitis, acute pancreatits, hyperplasia of Peyer's patches, and acute parotitis(Kaur et al., 2022).	Antibiotics

Thrombotic thrombocytopenic purpura (TTP)(Biswas et al., 2022)	It is idiopathic or autoimmune, but dengue progression can worsen the situation by deranging the liver enzyme and total bilirubin	Treatment with serial plasma exchange therapy, steroids, and monoclonal antibodies such as rituximab(Biswas et al., 2022)

On the other hand, a recent studies have shown that T-cells play a critical role in the pathophysiology of dengue fever. In particular, CD8+ T-cells have been found to be important in the control of dengue virus infection. However, excessive activation of these cells can also contribute to the development of severe dengue fever (Mathew, et al. 2014). Also, in dengue, it is uncertain how pathogen molecules, such as bacterial DNA, lipopolysaccharide (LPS), and serum (13)-D-glucan (BG), get from the stomach to the bloodstream. According to this study, elevated Proteobacteria (pathogenic bacteria from blood bacteriomes) as well as elevated endotoxemia and serum BG (leaky gut) were linked to an increase in monocytes, NK cells, and cytotoxic T cells as well as further influencing the immunological response to the dengue virus (Chancharoenthana, W, et al., 2022). These new findings provide a better understanding of the complex pathophysiology of dengue fever and may lead to the development of new treatments and preventive measures.

Chapter 4: Diagnosis of Dengue Fever

Due to the lack of a therapy or vaccine that is consistently effective, early identification of the dengue virus is crucial. At the moment, viral isolation, ELISA, and RT-PCR are the only methods available for diagnosing DENV (Dengue virus). These techniques allow for a precise diagnosis of dengue, but they are laborious, costly, and require trained personnel to execute (Hussain et al., 2022).

However, diagnosis can be classified into two distinct categories, which are clinical and laboratory diagnosis methods. The patient's clinical presentation may be a major factor in how difficult it is to make a dengue clinical diagnosis. The clinical spectrum associated with dengue infection may be similar to that of other infectious diseases in certain parts of the globe. Diagnostic biomarkers investigated in labs include the virus particle itself (separation in culture or vectors, or greater accuracy of viral genomic RNA), viral components (collection and identification of the released NS1 protein), and the host's immune response to viral infection (measured of virus-specific immunoglobulin M [IgM] and immunoglobulin G [IgG]) (Muller et al., 2017).

4.1 Clinical Diagnosis

The difficulty of making a clinical diagnosis of dengue might greatly depend on the patient's presentation and the phase of the disease. There is a broad range of disease-causing pathogens or illness states that might mimic the disease spectrum that is caused by dengue infection. This similarity is due to the fact that dengue infection can occur everywhere on the planet (Muller, Depelsenaire, and Young, 2017). Dengue is characterized by a short acute onset, headaches (usually behind the eyes), joint and muscular problems, and itchiness. However, it can vary in severity from a simple infection to dengue shock syndrome

(Chaloemwong et al., 2018). There are three stages to an infection's progression: febrile, critical, and recovering (Chawla, Yadav, and Chawla, 2014). The available literature for any diagnostic or laboratory indicators is therefore insufficient, despite the fact that alerting symptoms are thought to be a crucial part of rapid diagnosis of a possibly fatal illness (Jaenisch et al., 2016). For efficient patient care, accurate viral identification and assessment of warning indicators of illness before they advance to severe disease are essential (Muller, Depelsenaire, and Young, 2017).

Table 4.1: Phases of Dengue Infection at Clinical Settings

Clinical Phases	**Sign & symptoms**	**Duration**	**Detection Method**	**Complications**
Febrile phase	High fever associated with generalized pain and headache(Chawla et al., 2014) face flushing, skin erythema, generalized body soreness, myalgia, arthralgia, retro-orbital eye irritation, light sensitivity, rubeliform exanthema, and headache are some of the symptoms that may be experienced. (Muller et al., 2017)	less than 7 days(Chaloemwong et al., 2018) (Chawla et al., 2014)	(CBC), serological test or blood culture(Chaloemwong et al., 2018) Tourniquet test(Muller et al., 2017)	Dehydration, febrile seizure(Sarana, n.d. 2020)
Critical phase	Individuals whose capillary walls are more permeable to outside substances(Sarana, n.d. 2020) May show up as the warning symptoms (cold skin, hypotension, delayed capillary filling, low blood pressure, low volume, etc.). symptoms (lower urine production, discomfort in the middle and upper abdomen, a feverish face, a warm spine, cold and clammy limbs, and a hot flush)(Guzman et al., 2016)	usually on days 3–8 of illness (lasts 24-48 hours)(Sarana, n.d. 2020) and followed by a rapid convalescence(Guzman et al., 2016)	ultrasound detection(Muller et al., 2017)	Brain damage, irregular heartbeats, ventricular tachycardia, and even myocarditis and encephalitis may occur(Guzman et al., 2016) Less than five percent of patients have shock (DSS) or bleeding

				(DHF)(Chawla et al., 2014)
Recovery phase	Constant scratching and a slowing pulse. A fluid overflow situation may induce a loss of consciousness or convulsions, and skin rash or peeling might be a symptom. Tiredness might linger for many days.(Chawla et al., 2014)	48-72 hours followed by critical phase(Sarana, n.d. 2020)	IgM and IgG by ELISA(Simmons et al., 2015)	Syndrome of Acute Pulmonary Edema and Hypervolemia (Sarana, n.d. 2020)

4.2 Laboratory Diagnosis

In order to deliver effective and specialized clinical treatment as well as reliable public health monitoring, an accurate laboratory diagnosis of dengue infection is necessary. At the moment, the serological, virological, and molecular diagnostic approaches are the ones that are utilized most often for dengue detection (Subedi and Taylor-Robinson, 2014). It is necessary not only for the monitoring of the illness but also for the clinical treatment of the condition (Bhat et al., 2015). Also, if the cause of the fever is misdiagnosed as something else, such as malaria or pneumonia, treatment may be inefficient and costly (Hussain et al., 2022). Due to this, significant advancements have been made in the past few years in the improvement of diagnostic processes for specifying and diagnosing dengue infections that are available, cost-effective, concise, rapid, and rapid detection processes (Hegde and Bhat, 2022). The presented review paper examines a variety of tried-and-true methods for determining the presence of dengue fever, as shown in Table 3.

Table 4.2: Different diagnostic methods of dengue performed at laboratory settings

Diagnosis Methods		**Advantages**	**Disadvantages**	**When to Use**	**Sensitivity**	**Specificity**	**Biomarkers**
Serological	IgG antibodies assay by ELISA	Used this to determine if a case of dengue is primary or secondary(Bhat et al., 2015)	Requires a second blood draw, making it impractical for use in normal clinical treatment.	3-6 months after 4-5 days of onset	81.2% (Kabir et al., 2021)	39.8% (Kabir et al., 2021)	IgG
	IgM antibodies assay by ELISA	Patients in convalescence can be recognized by their IgM levels, which can rise as early as day 3-5 after original infection, peak several weeks following recovery, and then stay detectable for many months(Guzman et al., 2016)	Sample is required at the convalescent stage In less than 2% cases false positive reaction can be seen(Chawla et al., 2014)	Can be detectable for life after 4-5 days of onset	90%(Chaloemwong et al., 2018)	93% (Chaloemwong et al., 2018)	IgM
	NS1 antigen test	Can detect DENV NS1 in its acute phase, when it remains in the bloodstream for far longer than viremia does(Kabir et al., 2021)	One of the primary drawbacks of the NS1 detection approach is that it cannot identify the infection in samples taken during the late phase of dengue.(Bhat et al., 2015)	Between day 1-9 after fever is first detected(Subedi & Taylor-Robinson, 2014)	76% (Chaloemwong et al., 2018)	98% (Chaloemwong et al., 2018)	NS1 antigen

Virolo-gical	Virus Isolation	Highly specific result	Constraints on supply and high price (Bhat et al., 2015)	1-2 weeks (Bhat et al., 2015)	Mosquito cell lines' susceptibility to different strains of the virus varies(Chawla et al., 2014)	-	Virus Cell
Molecular	PCR-based test kits	The ability to detect dengue fever quickly and accurately at the earliest stages of infection	Price of the diagnostic kits; Utilizing the PCR-based procedures, it is possible to create false positives in vitro. Additionally, performing this manual assay needs trained individuals and laboratory facilities. (Kabir et al., 2021)	Can isolate dengue viruses as late as 10 days after symptoms appear.	25% to 100%(Bhat et al., 2015)	High Specifity	RNA

4.3 Newer Methods in the Diagnosis of Dengue

At the moment, it is important to create advanced point-of-care (POC) technology that can be used in a wide range of healthcare settings and is also reliable and cheap. This marketplace has a wide variety of technologies that can be used in all of the above situations. In addition, the existing commercial diagnostic procedures are not only prohibitively costly but also require specially qualified staff, making it impossible for devices to be transferred to less developed regions. These approaches are very sensitive, enable quick detection, can differentiate between serotypes, and may be utilized to diagnose dengue in a repeatable

manner. Nevertheless, the detection sensitivity differs depending on the serotype. The use of expensive gear, the necessity of temperature-controlled settings, and an operator with a high level of expertise are the three factors that limit the capabilities of PCR detection. These procedures need a complicated laboratory setup and have the potential to readily contaminate the material. In addition to this, the degree of sensitivity that diagnostic kits possess varies depending on the particular DENV serotype that is being tested for. It is possible that the discrepancies in the sensitivity of diagnostic devices against various serotypes are connected to the distinct NS1 epitope of different strains and the varied origin of the same serotypes, as the sensitivity of the Platelia test was poorest when compared to other serotypes for DENV-2 (Kok et al., 2023). Research has been conducted in order to bring about new cutting-edge technologies for improved specificity in the detection of the dengue virus in response to the constraints that are now associated with the diagnostic procedures that are in use.

Table 4.3: New cut-age diagnostic technologies of dengue

Diagnosis methods	**Uses**	**Advantages**	**Disadvantages**	**References**
Quartz Crystal Microbalance (QCM)	The technique is less time-consuming, cheaper, and less labor-intensive than conventional methods, and it does not require the use of any labels. Device's detection range for NS1 serum protein is 0.01-10 ng/mL.	Low-cost manufacturing, Super-fast analysis, and no labels needed, user-friendly	Delays in operation time, ineffective antigen recognition, interactions between natural antibodies, high costs, and difficulty in obtaining and preparing samples.	(Hussain et al., 2022)
Surface Plasmon Resonance (SPR)	Refractive index shifts are detected by SPR as a result of the molecular interaction	Low-priced, label-free, real-time detection with simple sample prep, high	Nonspecific binding that reduces accuracy	(Hussain et al., 2022) (Kabir et al., 2021)

	between antigen and antibody, allowing for the quick recognition of DENV.	throughput, and improved sensitivity		
Electrochemical Impedance Spectroscopy (EIS)	Used as a Point-of-Care tool for the diagnosis of DENV in a variety of settings.	Detection over a broad range, detection without labels, cheap cost, and ease of use.	Complex experimental setup	(Hussain et al., 2022) (Kabir et al., 2021)
Surface-enhanced Raman spectroscopy (SERS)	It is possible to detect infectious diseases like Zika by using the SERS active Ag-Au bimetallic nanowire device, which specifically targets the DENV-4 serotype.	For DENV detection, no reagents nor labeling of ssDNA were required. low LOD	Lacks robustness and repeatability compared to expensive portable Raman readers	(Kabir et al., 2021)
Microfluidics-Based Sensing Technologies	Can be used to identify a broad range of determinants, including IgM/IgG antibodies, NS1 antigens, E protein, and RNA.	The quantity of reagent needed for real-time detection is low; therefore the process is very economical.	Getting samples ready for these kinds of testing may take a long time and need a lot of expert labor.	(Kabir et al., 2021)
Non-Conventional Microfluidic-Based Sensing Technologgies	Detecting different flaviviruses utilizing recombinant NS1 proteins	Allows parallel testing high throughput, Separate dual infections by identifying their stereotypical symptoms.	Expensive and time-consuming since they need expert testing personnel and special equipment.	(Kabir et al., 2021)
CRISPR-Cas based assays	Including a sensitivity of 1 copy/L, this approach can identify DENV straight from patient samples in less than 2 hours.	Quickness, improved sensitivity, and a reasonable price tag	Rquire an experienced test supervisor	(Kabir et al., 2021)

4.4 Findings of this Review

The mosquito-borne dengue virus attacks immune cells and produces inflammation. A few of the symptoms are fever, headaches, pain in the muscles and joints, and a rash. Dengue hemorrhagic fever, which can happen in severe cases, is marked by plasma leakage and coagulopathy. This can cause bleeding and organ failure. Platelets drop when you have dengue fever, which makes it hard for your blood to clot. In severe cases, people may need to get platelet transfusions. How bad the disease is depends on how well the virus and the patient's immune system work together. Dengue fever can have a wide range of symptoms, and it can be hard to tell if someone has it because some of the symptoms overlap with those of other fever-causing illnesses. Additionally, T-cells play a critical role in the pathophysiology of dengue fever, and elevated Proteobacteria and endotoxemia can lead to an increase in monocytes, NK cells, and cytotoxic T cells, influencing the immunological response and have the potentiality to worsening the disease manifestation.

Clinical, epidemiological, and laboratory criteria are used to diagnose. There are serological, virological, and molecular ways to test for dengue fever in the lab. Serological tests look for the immune system's antibodies, while virological tests look for the virus in blood or other body fluids. Molecular diagnostic tests, like RT-PCR and LAMP, can find the virus's genetic material. These tests are very sensitive and specific, so they can diagnose a virus quickly and correctly. The lab test is a very important part of managing and treating the disease. But at this modern times, there are a lot of new ways to test for dengue fever, which shows that this virus could be found and treated sooner. According to my review and relevant findings, Surface Plasmon Resonance (SPR) is the most effective of these technologies that are used for the diagnosis of dengue. It is a highly sensitive, label-free method that can find interactions between biomolecules in real time. It has been used to quickly and accurately

find dengue virus particles and antibodies in serum samples. Other technologies include the Quartz Crystal Microbalance (QCM), which is a label-free method for detecting changes in mass on a sensor surface; the Electrochemical Impedance Spectroscopy (EIS), which can measure the impedance of an electrochemical system and has been used to find dengue virus particles and antibodies in serum samples; and the Surface-enhanced Raman spectroscopy (SERS), which is a highly sensitive and selective method for detecting molecular vibrations of an SPR, QCM, and SERS are all technologies with high sensitivity and specificity, but they can be expensive and need specialized equipment. Microfluidics-based sensors and CRISPR-Cas assays can be cheap and easy to move around, but they may not be very sensitive or specific. In the end, the best way to increase the accuracy of a diagnosis is to use a mix of different diagnostic methods.

For an accurate dengue fever diagnosis, a mix of these laboratory tests is needed since each has pros and cons. Diagnoses of dengue fever need to be made quickly, especially in places where it is common, so that patients can get the care they need and public health measures can be put in place.

Chapter 5: Conclusion

It has been a public health concern for some time that DENV has spread widely. Dengue fever is caused by the interaction between the dengue virus and the immune system of the person who has it. This interaction sets off a complicated chain of events that lead to the disease's symptoms. The pathophysiology of dengue fever can be distinguished by plasma leakage, thrombocytopenia, and hemorrhagic indications. Also, the diagnosis of dengue fever relies on the detection of the virus, viral RNA, or specific antibodies using various laboratory tests. However, these tests have limitations in terms of sensitivity and specificity, and there is a need for newer diagnostic approaches that can provide a more accurate and timely diagnosis of the disease.

This review paper summarizes the outlook into those newer methods with the aim of providing their list of resourceful information. Further studies are needed to make these technologies practical as rapid diagnostic tests for handling a future dengue outbreak.

The management of dengue fever is mainly supportive, and currently no specific antiviral treatment approach is available. Although treatment involves careful monitoring of patients for signs of severe dengue and prompt intervention to prevent complications such as shock and haemorrhage. Finally, dengue fever is still a worldwide national epidemic. Emphasis should be given to the need for ongoing study into the disease's biology and pathophysiology as well as the development of new diagnostic and therapeutic tools.

Chapter 6: Future Work

The spread of dengue fever is a major global health problem, and Bangladesh is no exception. In the past few years, there's been an increase in the amount of published research on the pathogenic mechanisms and diagnostic features of dengue fever, leading to new insights into the illness and more accurate tests for it. However, there is still much to be done in the field in terms of disease prevention, treatment, and management of this infectious disease. One important area of research that is likely to be the focus of future studies is the development of more accurate and sensitive diagnostic tests for dengue fever. While there are currently several diagnostic tools available, including PCR, NS1 antigen tests, and antibody tests, these tests are not always reliable, particularly in the early phases of the illness. More precise and sensitive diagnostic tools are required to identify the virus in its earliest stages of infection so that patients may get therapy sooner rather than later.

Another area of research that is likely to be a focus in the coming years is the development of effective vaccines against dengue fever. While there are currently several vaccines in development, there is still much work to be done in terms of determining the optimal formulation and dosing regimen, as well as ensuring the safety and efficacy of these vaccines. Proper research in these and other related areas is needed to find better ways to stop the spread of the dengue virus in developing countries like Bangladesh. This could include things like better sanitation and waste management, ways to get rid of mosquitoes, and public education campaigns about the disease and how to stop it from spreading.

In sum, although our knowledge of the pathogenesis and diagnosis of dengue fever has advanced significantly in recent years, there is still much more to learn and much work to be done to prevent, diagnose, and treat the disease. By focusing on areas such as diagnostic test development, vaccine development, and disease control strategies, researchers and public

health officials can work together to reduce the impact of dengue fever on communities in Bangladesh and around the world.

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