Project on

Dengue infection in Bangladesh: transmission, pathogenesis & control of dengue virus

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

The Department of Pharmacy,

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DECLARATION

I hereby declare that this project report, **"Dengue infection in Bangladesh: transmission, pathogenesis & control of dengue virus".** I am declaring that this Project is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Bachelor or any degree.

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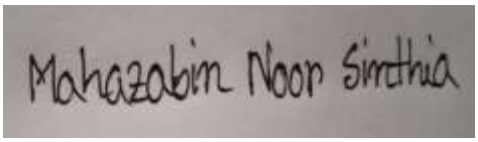

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I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

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Dedication…….

My Parents

The persons who always encourage me in every sphere of my life

Abstract

Dengue virus is a human pathogen that infects roughly 390 million people each year, with a quarter of them developing clinical symptoms. Although progress has been made in understanding the biology of dengue fever, there is still no licensed vaccine or antiviral medication available. There is yet no effective antidote for this virus. Patients' treatment is limited to symptomatic relief and Care that is supportive. As a result, the discovery of dengue treatments is critical. This The focus of this review is on the few compounds that have been tested in dengue virus patients: Balapiravir, chloroquine, lovastatin, prednisolone, and celgosivir are some of the drugs used to treat HIV. Balapiravir was expected that the medication would also be effective against DENV because the RdRp of DENV and HCV are comparable. The lessons that these have taught us Clinical trials can be extremely useful in the development of future trials for the dengue virus's next generation inhibitors.

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Chapter 1
Introduction

1. Introduction

The dengue virus (DENV) infection that affects 2.5 billion people in tropical and subtropical regions is the most prevalent and deadly mosquito-borne disease in the world [1, 2]. Infectious female Aedes mosquitoes, especially Aedes aegypti or Aedes albopictus, transmit DENV, a Flaviviridae family member, to humans [3,4]. The four dengue virus serotypes (DENV 1-4) share a lot of the same traits [5, 6]. Initial infection and secondary infection, two distinct types of diseases, can both be caused by DENV. The original infection that causes dengue fever causes it to be an acute febrile illness. The more serious results of reinfection are dengue shock syndrome (DSS) or hemorrhagic fever (DHF). [7]. Both DHF and DSS can cause patient death and have significant mortality rates [8]. As a result, dengue is regarded as a fever that carries a high risk of mortality. In addition, a vaccine that can protect against all four DENV serotypes must be developed and must be efficient, cheap, and safe. The dengue envelop protein facilitates DENV's binding to specific receptors, which causes the virus to begin its life cycle. The viral particle is merged into acidic lysosomes throughout receptor-mediated endocytosis after contact. The RNA eventually escapes into the host cell, where it controls the synthesis of viral proteins, and the viral particle separates from its coating. Within a few hours of infection, tens of thousands of duplicates of the viral molecules are produced from just one viral molecule, resulting in cell damage and, in severe cases, death. Seven of these proteins are produced and simplified, and three of them—the core protein, a surface protein, and an exterior protein—are structural proteins. The genes are arranged in the following order: NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3′ 5′-CprM (M) (Figure 1) [10]. Toll-like receptor-3, Melanoma Differentiation Related Gene-5, and Retinoic Acid irrational fear Gene-I are all thought to have played a role in the commencement of an effective IFN production contrary to DENV. This results from increased transcription of pattern recognition receptors both systemic and intracellular. [11]. Currently, there isn't a vaccine available to protect against dengue illness. Due to DENV's four distinct serotypes, developing a dengue vaccine has proven challenging. It is imperative to develop anti-dengue medications that are accessible, safe, and effective against all dengue serotypes. Heterochromatin was discovered for the first time 75 years ago. Soon afterward, silence genes were found to be present. This heterochromatin produces short RNAs through RNA interference that

regulate the modification of proteins and DNA in heterochromatic repetitions and transposable elements. [12]. Double-stranded RNA-mediated interference (RNAi) is a rapid and simple method to silence gene expression in a range of organisms [13]. RNAi and other silencing mechanisms are essential for regulating how cellular genes are produced in along with acting as a line of defense against entering viruses. Researchers showed that using RNAi to cure flavivirus infections in the host and stop flavivirus vector spread is a successful tactic. [14], [15].

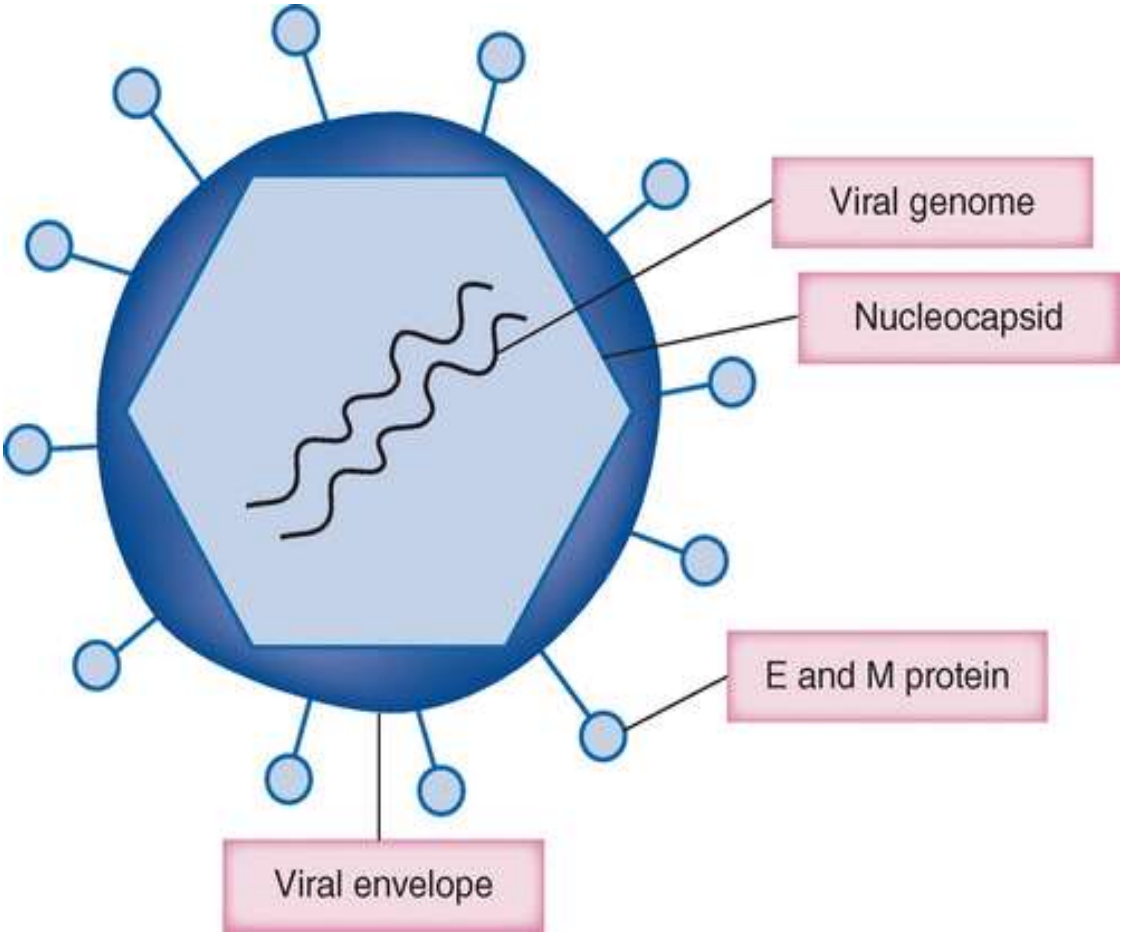

Figure 1: Structure of Dengue virus [16]

1.1 Dengue Transmitted to Humans

The dengue virus spreads through human-mosquito-human transmission in a closed loop. (Figure 2). [17] After being struck by an infected Aedes aegypti mosquito, viremia, or a condition where there is an elevated level of the dengue virus in the blood, typically develops four days later. Although it can last up to twelve days, viremia usually lasts five days. [18] On the first day of viremia, the patient frequently shows no symptoms of dengue. A person develops symptoms of dengue fever five days after being struck by an infected mosquito. These ailments may persist for a week or longer. Once a mosquito has ingested the blood of an individual who has the virus, it develops into a dengue vector. [19] The mosquito must feed on blood during the period of viremia, when the infected person's blood has elevated dengue virus concentrations. After ingesting the virus during the blood meal, the virus continues to propagate all through the mosquito's body for eight to twelve days. After this point, the infection can keep transmitting the dengue virus to another individual while being fed. [20] Does a mosquito carrying dengue merely transmit the disease to the next person it bites? No, a dengue-infected mosquito will continue to transport the virus throughout its entire life. Healthy people can acquire the dengue virus for the rest of their lives, which is usually a three to four-week period, from infected mosquitoes. [21] Both male and female mosquitoes ingest plant nectars, fruit juices, and other plant sugars as their main source of fuel. Therefore, why do mosquitoes attack humans? Since they require blood in order to lay their eggs, female mosquitoes bite people. Prior to laying a lot of eggs, Aedes aegypti frequently consume a lot of blood meals, and each female mosquito can produce multiple batches of eggs over the course of her lifetime. [22] The salivary glands of sick female mosquitoes contain the dengue virus. How does the virus spread from a mosquito's salivary glands to humans? throughout a blood meal, an infected female mosquito injects saliva into the human host to prevent blood clotting and to make feeding easier. [23] This saliva infusion allows the dengue virus to enter the host. Is the only way the dengue virus is transmitted to humans by mosquito bites? Rarely, organ transplants or blood transfusions from sources of contamination can transmit dengue. [24] There is evidence that a pregnant lady with dengue can transmit the disease to her unborn child. Mosquito bites are the main way that dengue illnesses are spread, despite these rare instances. [25]

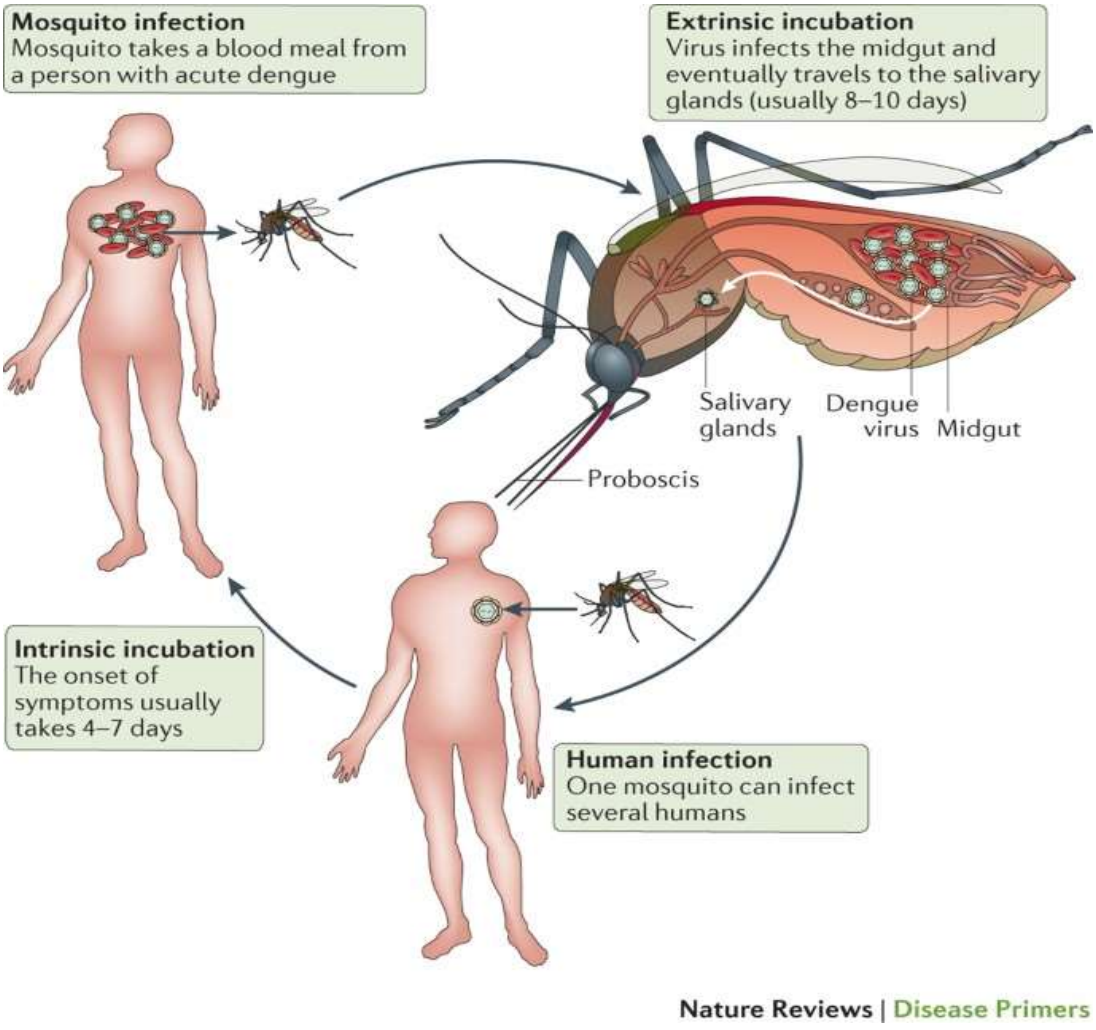

Figure 2: dengue virus cycle in humans and mosquito [26]

1.2 Dengue Mosquito Life Cycle

The life stages of mosquitoes are complex. (Figure 3). As they develop, mosquitoes change how they look and how they live. Female mosquitoes usually lay their eggs just above the surface in enclosures with water. Some examples of these containers include tires, buckets, birdbaths, water storage jars, and flower planters. [27] When the receptacles fill with water, which typically happens after a torrential the mosquito eggs hatch into larvae. Due to their aquatic nature, the larvae are dependent on aquatic microbes for sustenance. The three phases that constitute growth are completed by molting, or shedding, the skin, which occurs in larvae. These pupal stages are referred to as the first through fourth instars. [28] When a larva reaches the fourth instar, when it is completely developed, it undergoes metamorphosis, changing into a new form known as a pupa, the mosquito's "cocoon"

evolution. At this point in its life cycle, the mosquito is also aquatic. After two days, the mosquito pupa matures and breaks through the epidermis of the adult mosquito. The adult mosquito can fly and no longer dwells in water. [29] Its surroundings are on ground. When it doesn't rain, what happens? Because of how the Aedes aegypti mosquito has developed, its eggs can withstand dry conditions for a very long time. [30] When placed in a dry chamber, mosquito eggs do not hatch until the space inside is filled with water. [31] Because of this adaptability, it is now very difficult to completely eliminate mosquito populations. Dengue can be more dangerous in heavily inhabited areas, despite the fact that outbreaks are more likely to happen where there are many people in close contact with many mosquito vectors than in more remote locations. [32] In subtropical countries like Indonesia, India, Brazil, Thailand, Sri Lanka, and Myanmar that experience tropical monsoon seasons, dengue infections are a serious public health concern. [33]

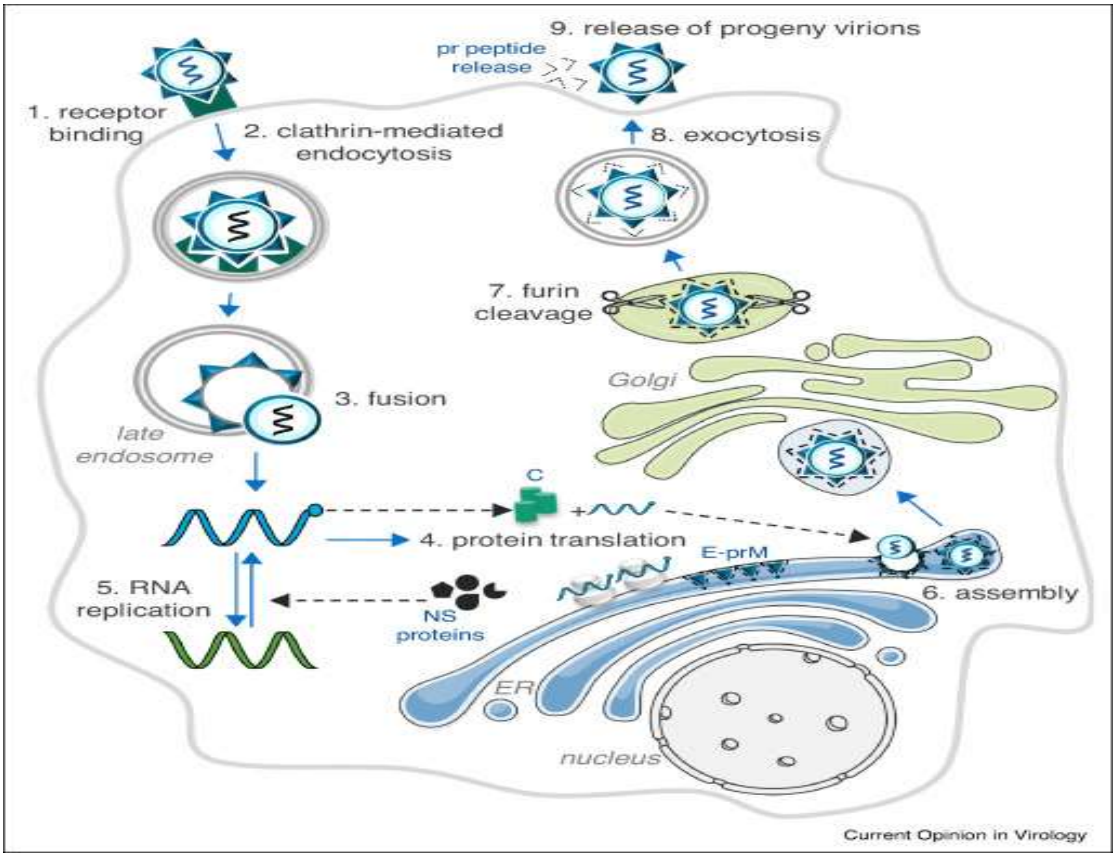

Figure 3: Aedes aegypti life cycle [34]

1.2.1 Epidemiology of DENV

When an Aedes aegypti or Aedes albopictus mosquito bite is the source of DENV, it can cause a variety of moderate to severe diseases. [35] The mosquito began to re-infest areas where it was previously eliminated as a result of the United States' program to eradicate it, which was discontinued in 1970. Aedes Aegyptus had a much wider geographic distribution in 2002 than it had before it was controlled and eradicated [36], which increased the number of dengue cases. DENV comes in four different serotypes. A single initial infection confers lifetime protection to a specific DENV genotype. [37]

1.2.2 Pathogenesis of Dengue virus

The pathogenesis of DHF/DSS, the most severe form of DENV infection, is complicated by a complex interaction between the human immune system and viral virulence factors. [38] because there is a higher chance of DHF with secondary DENV infection and in children born to DENV-immune mothers within the first year of life, controlled trials have demonstrated a relationship between the immune system and DHF. [39] The idea of particular antibodies immune augmentation (ADE) of infection was born as a consequence of these findings. Patients with significant secondary DENV infection had higher peak viremia, supporting the ADE pathogenesis hypothesis. The possibility of DHF was found to be higher when DENV infection in macrophages was amplified in vitro by antibodies. [40] Elevated amounts of cytokines like interferon (IFN), tumor necrosis factor (TNF), and interleukin (IL)-10 are associated with serious diseases. The extent of the sickness has been linked to the activation of CD8+ T cells and the emergence of serotype-reactive low-affinity DENV-specific T cells. [41].

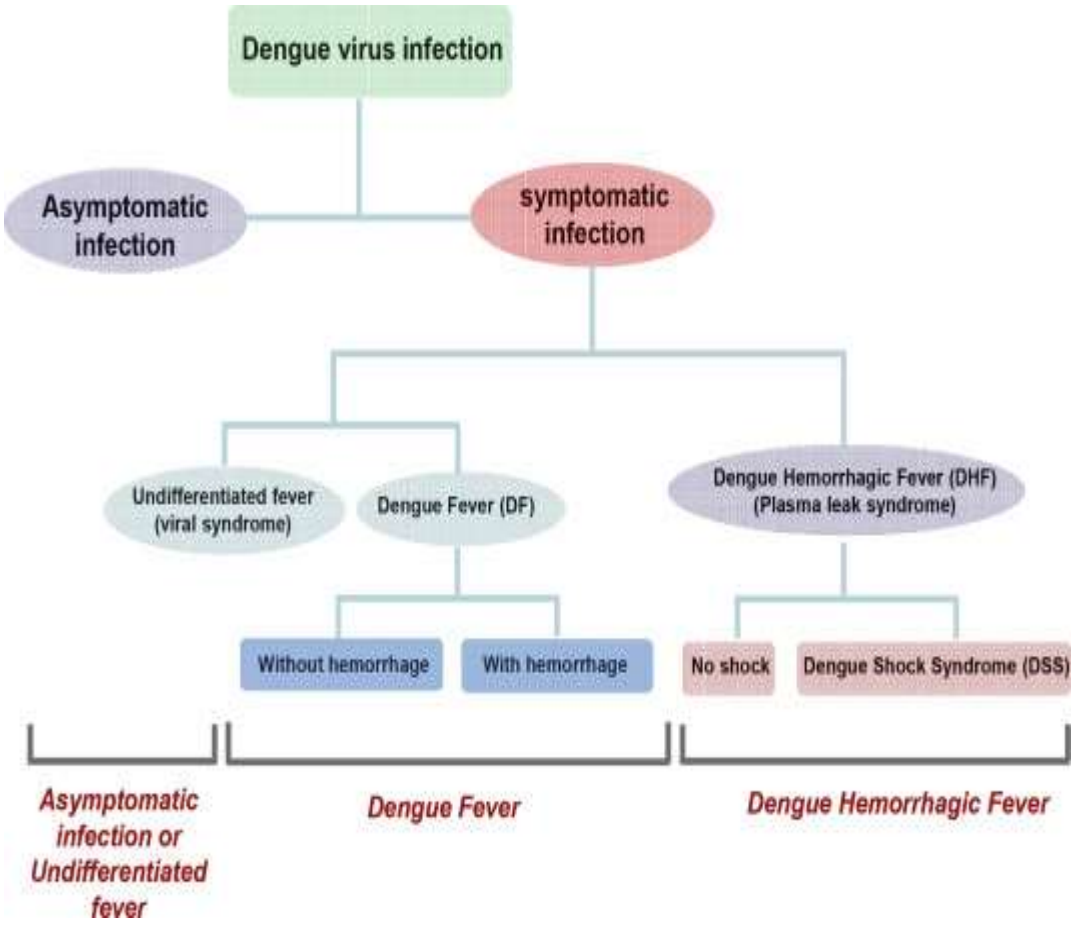

Figure 4: Pathogenesis of Dengue virus [42]

1.2.4 Diagnosis process for DENV

The creation of an infection diagnostic tool is necessary for dengue identification. Because dengue viruses can cause asymptomatic sicknesses and a variety of clinical symptoms ranging from a mild, generic febrile illness to a fatal hemorrhagic disease, monitoring, reaction, and case detection are difficult tasks. [43] Significant risk factors for DHF include the patient's age, immune function, genetic illness, virus strain and serotype, and immunological status. Usually, the virus is discovered through cell culture or serology, which scans for anti-dengue antibodies. Both particular dengue virus antigens and viral RNA can be found and grown in vitro. [44]

Chapter 2
Purpose of the study

2.1 Purpose of the study

- The goals of this project are to get a comprehensive thoughtful of the medical problem being researched.
- To learn more about the variables that subsidize to the expansion of Dengue virus infection.
- To have a better grasp of the many diagnostic measures used to detect this ailment.
- To gain a systematic considerate of the bug, as well as its cause, signs and symptoms, consequences, and medical and nursing management choices.
- The determination of this investigation was to recognize more about Dengue virus infection in the world.
- Designate the epidemiology of Dengue virus infection.
- Review the exhibition of a patient sick with Dengue virus infection.
- To discovery out permitted beneficial practice for Dengue virus infection.
- Recapitulate the role of the interprofessional healthcare team in Dengue virus infection illness preclusion and control measures.

Chapter 3
Methodology

3.1 Materials and Procedures

The methods employed in this investigation are discussed in this chapter. It is a explanation of the study environment. The study population, the study sample, the research equipment, the technique, and the data analysis are all factors to consider.

3.2 Research Methodology

This is a summary of prior studies on different clinical trials as a dengue virus disease treatment.

3.3 Inclusion and Exclusion Criteria

All studies on Drug candidates in clinical trials for dengue virus disease.

3.4 Data Collection Procedure

Data was gathered directly from prior study articles, while another portion was gathered through searching the internet for relevant information. The activities of many treatments were recorded.

3.5 Method of data analysis

All of the information gathered from prior study publications was numerically coded and imported.

Chapter 4
Results & Discussion

4.1 Vaccination

The development of a dengue vaccine presents unique challenges. The four dengue serotypes are widespread, and transmission to one of them confers lifetime immunity to that serotype of illness [46], but only temporary protection against the other three serotypes. Furthermore, dengue differs from other diseases in that there is a possibility of developing a serious and possibly fatal illness with different serotypes [77]. Little is known about how a virus interacts with the immune system or how certain types of inherent immunity can make a condition worse. In order to decrease the risk of vaccination recipients developing an extremely serious illness, a reliable and effective dengue vaccine should be tetravalent and provide substantial and long-lasting protection against all 4 serotypes simultaneously. [78]. The development of several dengue vaccine candidates is at different stages. In the more complex forms, tetravalent mixtures of live, attenuated viruses matching each serotype are used. Utilizing a variety of attenuation mechanisms, three of the top alternatives have been created: [47]

1) A single inoculation cannot defend against all four serotypes due to viral contact among some of the live vaccine components.

2) Booster doses given no more frequently than every six months are ineffective. [48] To generate adequate neutralizing antibody responses to all four serotypes, live attenuated vaccines must be given three times over the span of a 12-month dosage interval. As a result, if an infection develops between the first and last immunization, there is a possibility that the insufficient response brought on by the earlier vaccinations will make the sickness worse. [49]

Name of vaccine	Types of vaccine	Manufacturer
Dengvaxia	live attenuated tetravalent chimeric vaccine	Sanofi Pasteur
TAK-003 or DENVax	Recombinant chimeric vaccine	Mahidol University in Bangkok
TV-003/005	monovalent vaccines	NIAID
TDENV PIV	inactivated vaccine	GlaxoSmithKline (GSK) and the Walter Reed Army Institute of Research (WRAIR)
V180	recombinant subunit vaccines	Merck & Co., Inc. is an American multinational pharmaceutical company
DNA vaccines	monovalent DNA plasmid vaccine	Naval Medical Research Center

Table 1: Available Dengue vaccine

4.2 Balapiravir

Balapiravir is a prodrug of the nucleoside analogue 4′-azidocytidine (R1479), which is an effective inhibitor of the hepatitis C virus's (HCV) in vitro reproduction [50]. When the original substance enters the host cell and is phosphorylated to produce its 5′-triphosphate metabolite, it acts as a viral RdRp antagonist. (Figure 1). Patients with persistent HCV illnesses have demonstrated the effectiveness of balapiravir [51,52]. The research phase of this medication for the management of chronic HCV was halted due to an insufficient benefit to relative risk [53]. The drug's effectiveness toward DENV was anticipated given that DENV and HCV both have RdRps that are similar.

Balapiravir has been shown to inhibit the replication of a number of DENV serotypes and genotypes (both lab- and clinical-based) with EC50 values in the range of 1.9 to 11 M in human hepatoma (Huh-7) cells and 1.3 to 6.0 M in cells that originate from human macrophages and dendritic cells [54]. The antiviral activity was tested using peripheral blood mononuclear cells (PBMCs), and the EC50 values varied from 0.10 to 0.25 M [56]. A DENV management trial using oral medication doses of 1500 and 3000 mg BID was designed based on these measurable measurements. [57] This resulted in median plasma sensitivities of 3.56 and 5.85 M, respectively, which are 1.6 and 2.6 times higher than the EC50 value found in macrophages. [58]. Numerous causes have been suggested for the absence of potency. First off, when viremia is at its highest level, the medication might not be as efficient. Balapiravir was actually 52 times less effective in PBMCs when added after an in vitro infection started [59]. In Huh-7 and adenocarcinomic human alveolar basal epithelial (A549) cells and to a lesser extent in human monocytic THP-1 cells than in primary cells, additionally to being less active when introduced following infection, the action of balapiravir appears to be cell-dependent [60]. This may be due to differences in the ability of different cells to phosphorylate balapiravir, which results in the active triphosphate metabolite of the medication. [61]

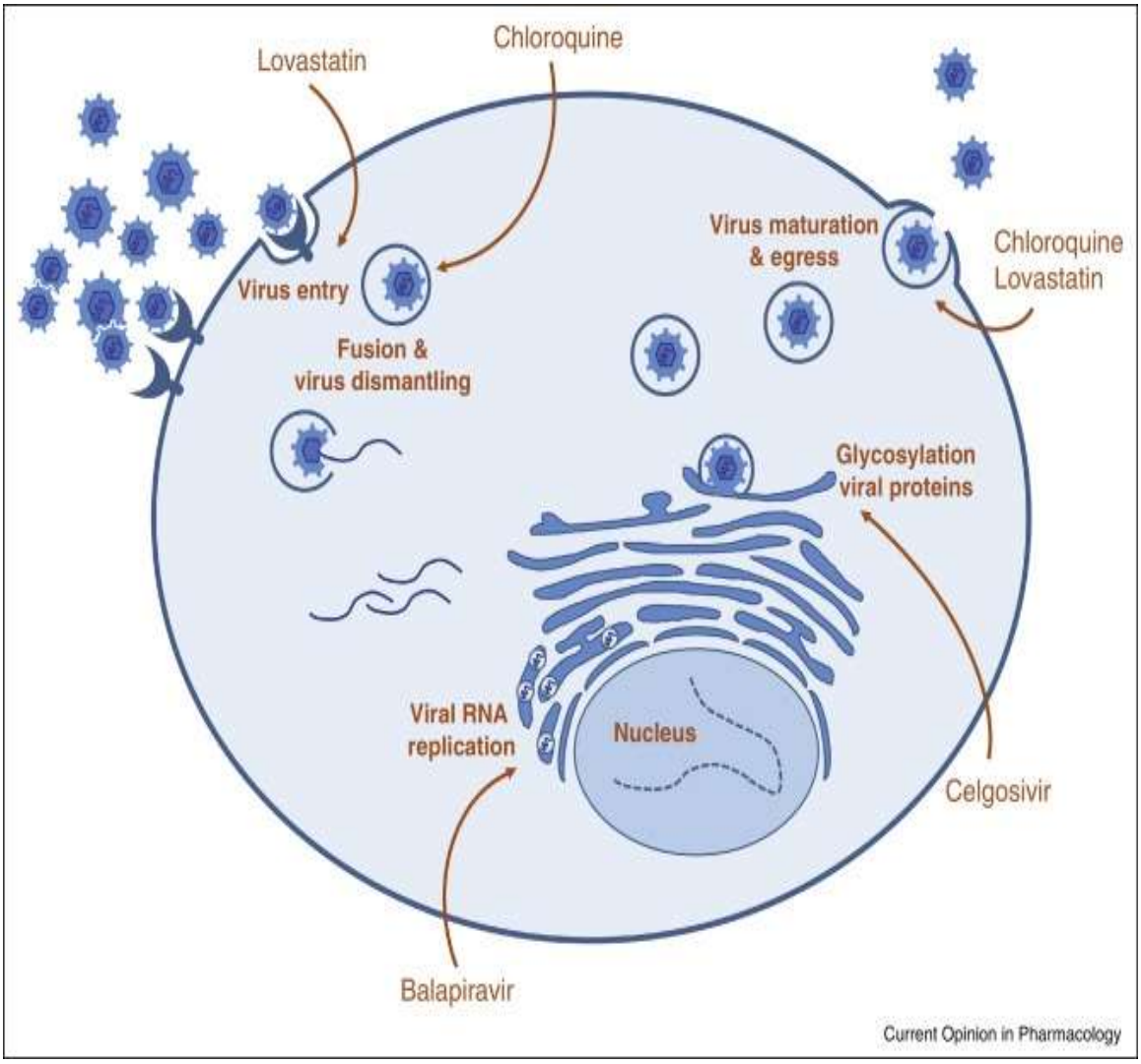

Figure 5: Mechanism of action balapiravir [62]

4.3 Chloroquine

Chloroquine is mainly used to treat and prevent malaria in areas where the disease is still treatable. [63] Complex instances, vulnerability testing, and particular types of malaria frequently necessitate the use of additional or complementary medications. [64] Other conditions that might react to chloroquine therapy include rheumatoid arthritis, lupus erythematosus, and amebiasis that spreads outside of the intestines. [65] Although it hasn't been thoroughly researched throughout pregnancy, it seems secure. [66,67] It was tested to treat COVID-19 early in the pandemic, but these experiments were largely discontinued in the summer of 2020, and it is not recommended for this use. [68] It's taken directly. Common side effects include skin rashes, diarrhea, lack of appetite, and muscle problems.

[69] Serious side effects include vision problems, muscle injury, convulsions, and low blood cell counts. Chloroquine belongs to the chemical group 4-aminoquinoline. [70] By preventing the asexual step of the malaria parasite's life cycle within the red blood cell, it functions as an antimalarial. [71] It's unclear how it works in rheumatoid arthritis and lupus erythematosus. [73] Chloroquine was discovered in 1934 by Hans Andersag. [72] The Health Organization has named it as one of the Essential Medicines. [73] It is available as a generic medication.

4.4 Celgosivir

When paired with pegylated interferon alfa-2b and ribavirin, celgosivir has demonstrated a synergistic effect in both studies in vitro and in phase II clinical trials that last up to a year in patients with chronic HCV infection. [80] When used alone, celgosivir is useless in the treatment of HCV. Celgosivir may prove to be an essential component of conventional therapy and may help stop the emergence of drug tolerance. Long-term toxicity studies must be conducted to verify celgosivir's safety in people. [75] Celgosivir is usually well tolerated and safe, but it doesn't seem to affect the virus loads or frequency of fever in dengue fever patients. [76]

4.5 Drugs targeting dengue proteins

Numerous direct-acting antiviral drugs (DAAs) focus on specific dengue protein targets. It is advised that interested users peruse these excellent summaries on the most recent advances, chemical structures, and mechanisms of action of some novel therapeutic antibodies and DAAs [91–93], which will not be discussed in-depth here. Rather, we have offered comprehensive summaries of how various dengue-treatment methods have evolved. [78]

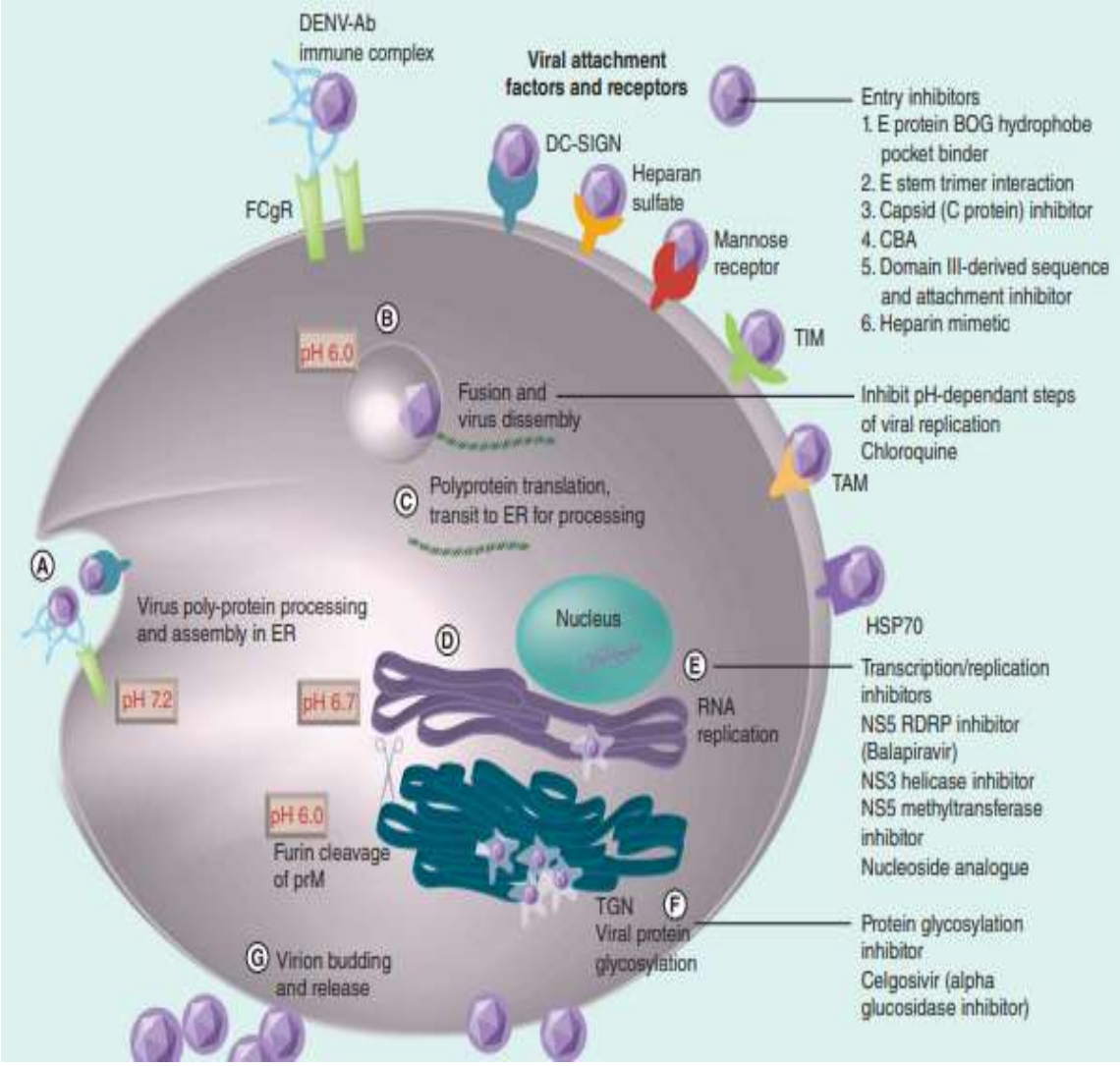

Figure 6: Schematic view of dengue virus replication cycle. [79]

4.6 MTase inhibitors

The NS5-MTase domain catalyzes RNA cap methylation at the N7 and 2'O sites on freshly synthesized positive-strand RNA [94]. In the viral RNA genome, it can also methylate the internal adenosine at the 2'-OH position of the ribose. Since MTase mutations that eliminate N7 methylation are fatal for flaviviral replication, N7 methylation of the RNA cap is required for effective translations [94]. New compounds (like "compound 10") that selectively bind to and inhibit MTase are still being created. For additional testing, suitable chemicals are currently being immovably isolated [95]. Nucleoside analog (NITD008) It

has been discovered that the adenosine analog NITD008 has antiviral effects by inhibiting DENV RdRP and RNA chain synthesis. [96]. NITD008 was found to prevent DENV 1-4 replications in vitro. NITD008 reduced viral loads, proinflammatory cytokines, and mortality in DENV-infected mice. This medication was not further refined for use in human trials because, however, preclinical toxicology tests in mice revealed substantial adverse effects. [96].

4.7 Helicase inhibitors

Helicase antagonists stop DENV NS3 from unraveling after RNA replication [97]. The small-molecule helicase blocker ST-610 was also discovered. All four DENV serotypes were successfully and precisely inhibited by this medication in vitro [98]. It effectively reduced viremia in rodents and was well absorbed. Its use is constrained, especially in environments with limited resources, due to its poor oral bioavailability and need for parenteral administration.

4.8 Protease inhibitors

The cofactor NS2B is required for DENV NS3, a serine protease, to function. Viral replication requires correct co- and post-translational processing of the polypeptide when DENV replication, which produces structural and nonstructural proteins [99]. Numerous proteases (NS2B/NS3) produced by the host and virus are required for these stages. The extremely potent antagonists of HIV-1 and HCV proteases show that viral proteases are established antiviral targets [99]. It could be done to create proteolytic enzymes that are effective toward all four DENV serotypes given that the DENV 1-4 NS3 protease shares 63–74 percent of its amino acid sequence, but the barrier to protection may be minimal. Despite the discovery of some possible antagonists, these medications have not yet undergone human clinical trials. [92].

4.9 NS4B inhibitor

The transmembrane protein NS4B, which has no known enzymatic activity, is necessary for the formation and binding of the active viral replication complex to ER membranes [100]. In order to encourage viral replication, NS4B blocks IFN-/ signaling [101]. To stop DENV replication, many researchers have discovered substances that interfere with NS4B. One of the substances discovered and shown to be efficacious in contradiction of all four

DENV serotypes was NITD618 [102]. Though, NITD618 makes in vivo experiments difficult because of its poor pharmacokinetic features and high lipophilicity [92]. Another NS4B antagonist was lycorine, a plant alkaloid that stopped WNV, YFV, and DENV 1-2 from replicating [103]. SDM25N, a different substance, was found to be efficient against DENV 2 in vitro.

4.10 Entrance/fusion blockers

Three cysteines found in the E protein are crucial in enabling DENV entrance into the host cell. domains (DI, DII, and DIII) [84]. The dimer association occurs in the enhanced E protein, which is organized into 90 DENV particles homodimers with a covert fusion loop. When viral E protein binds to numerous cellular receptor and/or connection types factors (like CD14, GRP78, laminin receptor, mannose receptor, DC-SIGN, heparin sulfate, and TAM proteins), this marks the beginning of a virus entry [85]. binding is completed, and clathrin endosis controls viral uptake [85] Because of the acidic pH environment inside the cell, the E protein experiences structural modifications and combinations that expose the fusion loop and cause it to be inserted into the endosomal membrane. The next step is the formation of a membrane fusion pore, which allows viral RNA to enter the cytoplasm. Recently suggested membrane fusion antagonists have been developed [85]. They attach to various parts of the structural framework. Prior to their implementation, further optimization, animal, and clinical studies are required. [82].

4.11 DNA-based analog (NITD008)

It has been established that the adenosine analog NITD008 has antiviral properties because it inhibits DENV RdRP and halts the synthesis of RNA chains [90]. In vitro studies showed that NITD008 reduced DENV 1-4 replication. DENV-infected mice treated with NITD008 displayed reductions in viral load, proinflammatory cytokines, and mortality. Since this medication also had significant side effects during preclinical toxicology studies in mice, it was never investigated for human trials. [90].

Name treatments	Mechanism
Balapiravir	inhibitor of the viral RdRp
Chloroquine	constraining the entry of (dengue) viruses
Lovastatin	block a variety of biological mechanisms
Prednisolone	Corticosteroids may reduce symptoms
Celgosivir	inhibits DENV1-4 clinical isolates' in vitro replication
cromolyn, montelukast, and ketotifen	Mast cell inhibitors
Statins	(HMG-CoA-reductase inhibitors)
Drugs targeting dengue proteins	dengue proteins inhibitor
flaviviral	MTase inhibitors
DENV NS3	Helicase inhibitors
thyrothricin	Protease inhibitors
Others	NS4B inhibitor

Table 2: Available Medication for dengue virus

Chapter 5
Conclusion

5.1 Conclusion

Dengue poses a threat to world health because it affects over 2.5 billion people. For reasons that are still not completely understood, the number of dengue cases has dramatically increased over the past 50 years. Even though it is a short-term condition with minimal long-term consequences, a severe infection can be fatal. Although the pathophysiology of dengue is still unclear, it is generally accepted that the "severe disease" is brought on by an interaction between the virus and the host's excessively responsive immune system. The only form of treatment for the illness is palliative treatment, along with meticulous fluid management during the critical phase and ongoing observation. The benefits of a dengue vaccine are enormous, yet progress in this area is delayed because of numerous technological challenges.

Chapter 6
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