EPIDEMIOLOGICAL STUDY OF DENGUE VIRUS INFECTION IN HOSPITALIZED PATIENTS

Name: Shirajum Monira

Roll no.: 0119/01

Registration no.: 728

Session: 2019-2020

A thesis submitted in the partial fulfillment of the requirements

for the degree of MPH (One Health)

One Health Institute

Chattogram Veterinary and Animal Sciences University

Chattogram-4225

June 2020

Authorization

I hereby declare that I am the sole author of the thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize the CVASU to reproduce the thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

Shirajum Monira

June 2020

EPIDEMIOLOGICAL STUDY OF DENGUE VIRUS INFECTION IN HOSPITALIZED PATIENTS.

Name: Shirajum Monira

Roll no.: 0119/01

Registration no.: 728

Session: 2019-2020

This is to certify that we have examined the above MPH (One Health) thesis and have found that is complete and satisfactory in all respects, and that all required by the thesis examination committee have been made.

----------	**----------**
Prof. Mohammad Mahmudul Hassan, PhD	**A/P Shahneaz Ali Khan, PhD**
Supervisor	**Co-supervisor**

Prof. Sharmin Chowdhury, PhD

Chairman of the Examination Committee

One Health Institute

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

June 2020

Acknowledgements

All praises to almighty Allah who gave me the opportunity to be enrolled in the **One Health Institute** for achieving Master in Public Health. I would like to express my veneration to honorable supervisor Professor **Dr. Mohammad Mahmudul Hassan**, Head, Department of Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU) for his coherent and articulated instructions. It would not be possible to complete such a laborious task without his scholastic guidelines. It was an exquisite experience for me to work under his supervision. I feel much pleasure to convey my gratitude to honorable co-supervisor Dr. Shahneaz Ali Khan for his valuable suggestions and inspiration. I am grateful to Dr. Mohammad Ezazul Hoque Iqubal, Lecturer, Department of Pharmacology, Enam Medical College for his support in relation to data collection and for arranging all institutional support regarding laboratory testing and for fulfilling all other institutional requirements. Special thanks to him for his encouragement and cordial co-operation throughout the work. I would like to acknowledge the support and encouragement received during MPH program from other teachers, technical and non-technical staffs of the One Health Institute, CVASU.

I am also grateful to my father Mr. Shafique Ahamed, my mother Mrs. Maidun Naher and family members for their allowance and heartiest support.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Elaboration
ADE	Antibody dependent enhancement
CF	Complement Fixation
DENV	Dengue Virus
DF	Dengue Fever
DGHS	Directorate General of Health Services
DHF	Dengue Haemorrhagic Fever
DIC	Disseminated Intravascular Coagulation
DSS	Dengue Shock Syndrome
EDS	Expanded Dengue Syndrome
EIP	Extrinsic Incubation Period
ER	Endoplasmic Reticulum
hCF	Human Cytotoxic Factor
ICT	Immune-chromatographic Test
IEDCR	Institute of Epidemiology Disease Control and Research
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IHR	International Health Regulations
IVM	Integrated Vector Management
LMICs	Lower and Middle Income Countries
MNL	Mononuclear Leucocyte
NS	Non Structural

NT	Neutralization Test
PAHO	Pan American Health Organization
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription polymerase Chain Reactions
SEA	South East Asian
SA	South Asian
TDV	Tetravalent Dengue Vaccine
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
UD	Unusual Dengue
UF	Undifferentiated Fever
UHC	Upazila Health Complex
VBDs	Vector Borne Diseases
WBC	White Blood Cell
WHA	World Health Assembly
WW	World War

Abstract

An Arboviral infection, Dengue Fever (DF), which has emerged as its two severe forms including Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) is found in tropical and subtropical regions around the world. It has shown a massive global increase in frequency of epidemics along with an increase in disease incidence over the past three decades predominantly in urban and peri-urban settings. This study was conducted to assess the sero-epidemiology of dengue viral infection among the clinically suspected patients admitted in two medical facilities such as Enam Medical College (EMC) and Savar Upazila Health Complex (UHC)) in Savar, Dhaka during January to December 2019. Blood samples were collected from total 449 suspected patients and separated sera were tested for NS1 dengue antigen, anti-dengue IgM, and IgG antibodies using commercially available diagnostic test kits. Out of 449 patients 329 (73.27%) were serologically positive for dengue specific antigen and antibodies. Among them, 136 (60.7%) from EMC and 166 (73.8%) patients from UHC were found positive for Dengue NS1 Antigen. Total 28 were positive for anti-dengue IgM and IgG antibodies test and among them only 2 patients showed recent infection. IgM positive dengue patients were 5.8% in EMC and 6.7% in UHC. The percentage of IgG positive dengue patients from both hospitals were similar and it was 5.8%. This study represents the presence of Dengue virus in Savar Upazila of Dhaka district and corresponds one of the few studies to evaluate the sero-prevalence of Dengue virus in Bangladesh. A substantial percentage of suspected patients in this study was negative by dengue tests suggesting other infections of similar clinical presentations that are becoming prevalent in the study area.

Chapter 1: Introduction

Dengue ("pronounced DEN-gi") is one of the most rapidly spreading febrile illness caused by a Flavi virus transmitted by mosquito vectors (Davidson, 2018). It has become a global burden since the Second World War and this disease is eminent in more than 120 countries globally. The geographical distribution showed that this virus encountered the population in America, South East Asia (SEA) and Western Pacific regions; with Asia representing 70% of the global load of disease (WHO, 2020). Approximately 400 million infections and 100 million clinically apparent infections occur in each year (Davidson, 2018). *Aedes aegypti* mosquito is the principal vector of transmitting Dengue virus, which lives in the close proximity of urban settings and prefer to breeds mostly in artificially congregated fresh water. The mosquitoes are preferably daytime feeders, with peak biting periods being observed early part of the morning and just before the dusk. A secondary vector of the virus is *Aedes albopictus*, an Asian ancestor mosquito that can tolerate lower temperature than *aegypti* and has spread to more than 32 states of USA and more than 25 countries of European region with the expand of international trading of used tires and other goods (WHO, 2020). The virus utilizes humans as the ultimate disease producing reservoir host, but a jungle cycle involving monkeys as the reservoir and other *Aedes* species as vectors is suspected (Levinson, 2016). This virus has four serotypes that currently circulating including DENV-1, DENV-2, DENV-3, and DENV-4 with similar impact on host animals. Interestingly, Infection with one serotype confirms life-long antibody mediated immune response against that particular serotype but this immune protection does not persist extended period against different serotypes (Yung et al., 2015).

Classical dengue infection begins with an influenza-like syndrome consisting of pyrexia, cough, headache, swollen glands, muscle pain, joint pain (break-bone fever) appeared 3-7 days after a patient being bitten by an infected mosquito (Mahmud, 2019) . However, in severe form of infection abdominal pain, bleeding gums and rapid breathing been observed in affected patient. In recent time, some of the designated signs were more or less absent from the patients but more severe symptoms appeared much earlier than normally expected which could confuse health care providers to determine the disease in initial stage (Mahmud, 2019). The striking issue of this severe form of infection is the fatality rate that approaches around 10%.

DHF occurs particularly in Southern Asia, where as the classic form is commonly found in topical areas worldwide (Levinson, 2016). Individuals who are immune to one serotype and then become infected with another serotypes due to an anamnestic, heterotypic response elicits cross-reacting antibodies in higher concentrations against the first serotype (Levinson, 2016; Davidson, 2018). In the initial period dengue was frequently seen in children >2 years old and DHF/DSS in children 2-15 years old. But recent studies show that these conditions are now also affecting children <2 years old and most commonly in 16-45 years age group or older (Davidson, 2018). Patients who develop significantly low blood pressure can have fatality rate of up to 26% (Ranjit and Kissoon, 2011). Children <5 years old have 4 times greater risk of death than among those over the age of 10 (Kularatne, 2015).

No specific treatment regime has been developed for dengue fever (Whitehorn and Farrar, 2010). Most of the patients being treated with the aid of symptomatic drugs. Depending on the symptoms, some people can get better only by drinking fluids at home, whereas some people need hospitalization (CDC, 2019b). From vaccine point of view, there is only one licensed vaccine (Dengvaxia from Sanofi pharmaceutical company) that has been formulated for administrating over 9 years aged group population of Bangladesh and has only about 65% efficacy (Mahalingam et al., 2013). This is a live attenuated tetravalent vaccine consisting chimeras which made up of structural pre-membrane (prM) and envelope (E) genes of the four dengue virus serotypes along with non-structural genes of Yellow Fever 17 D vaccine strain (chimeric Yellow Fever Dengue –CYD). This vaccine recommended to be given in three divided doses scheduled six month apart; 0, 6 and 12 months. This vaccine produce antibody responses with against DENV specific cellular response that has been demonstrated following a natural infection (Thomas and Yoon, 2019).Diagnosis of DENV infection depends on direct virus identification and serological tests. Serological tests detect humans-derived immune components that are produced in response to the infection. Commercially manufactured rapid diagnostic test kit is available for detection of a virus originated protein particle known as NS1 antigen that takes about 20 minutes to detect and does not need specialized lab techniques or equipment. Detection of IgM and IgG anti-dengue antibodies can confirm the presence of recent and past infection respectively. RT-PCR

can also be used for identify the genotyping of the virus that allow comparisons with the virus samples from various geographical sources (WHO, 2020).

The 2005 World Health Assembly resolution (WHA 58.3) on the revision of the International Health Regulations (IHR) has included dengue a disease that may emerge as a public health emergency of international concern having implications for health security due to disruption and rapid epidemic spread beyond national and international borders (WHO, 2012-2020). Throughout the tropics dengue is very much common, with risk factors influenced by local spatial variations of rainfall, temperature, relative humidity, degree of urbanization and quality of vector control services in urban areas (WHO, 2020). Some studies revealed that, from the epidemiological and entomological perspectives, climate change after 2014 might have caused some ecological imbalances in the environment that lead to occurrence of more dengue cases in the pre-monsoon season and had led to an explosion of Chikungunya for the first time in Bangladesh in 2017 (Mutsuddy et al., 2019). The CDC unit of DGHS warned about a major outbreak on March 2019 in Bangladesh. As per suspicion, a nationwide occurrence begun primarily on April 2019 and this phenomenon is still going on. Dhaka is the worst affected city in the country and the districts of Dhaka division are among the hotspot of this infection (Pokharel, 2019). Unfortunately, the virus diffuses into rural areas as well, as a significant number of people travelled from major cities to rural areas to celebrate Eid al- Adha holidays in August (BBC, 2019). It was demonstrated that, in 2019 cases of dengue fever became nearly doubled in comparison to the total from last 19 years (Hasan, 2019). Dhaka, a megacity of over 19 million inhabitants has thousands of under construction building sites, full of pits that can hold stagnant water during monsoon season that invites mosquitoes to breed. In addition, lack of public awareness, ineffective insecticides, lack of specific treatment and preventive measures are constantly giving an undermined threats for near future and ongoing outbreaks (Hossain et al., 2020b). This study provides specific answer on the epidemiological aspects of dengue based on analysis of demographic variables, serological prevalence of dengue, clinical and immunological data. It meets the current knowledge of burden of dengue, outcome and vulnerable group with a view to better controls and management of this infection. The availability of the comprehensive serological, clinical and demographic data can

be useful to the decision makers and public health officials in evaluating, controlling, managing and preventing dengue outbreaks efficiently.

Aim of the study:

Dengue has now become an emerging health threat in many countries of the world with the potentiality of causing pandemics. Scientists are now conducting researches regarding the mechanisms by which the dengue virus causes diseases by giving emphasize on understanding dengue pathogenesis, the virus itself and vector biology. Scientists are also working to improve diagnostics for patients with dengue so that effective treatment can be implemented to control the infection. Clinicians are then applying statistical modeling for precisely diagnose patients and can predict their prognoses by tracking clinical data, such as patients' platelet count, the presence of pre-existing IgG antibodies against dengue virus. Besides, improving the dengue surveillance for standardize reporting system of dengue cases is an essential way to prevent and control dengue transmission. Therefore, this study was undertaken to describe the frequency, distribution, sero-prevalence of dengue fever based on the variables such as age, sex, weight, clinical symptoms and serological evidence during the year of 2019 in Savar Upazila of Dhaka district. The specific aim of the study is

1. To estimate the burden of dengue viral fever in Savar Upazila

2. To assess the demographic information and spread of dengue over time at surrounding government and non-government hospitals in Savar

Chapter 2: Review of the literature

2.1: History of Dengue fever

A case of a probable dengue fever was first recorded in a Chinese medical encyclopedia from the Jin dynasty dating back to 992 BC (Salles et al., 2018) . In a report regarding the 1780 epidemic in Philadelphia (published in 1789); a physician and United States Founding father Benjamin Rush first applied the term 'break-bone fever'. He also used the more formal term 'bilious remitting fever' in the report title (Barrett and Stanberry, 2009). It was only after 1828, the term 'dengue fever' came into a regular use. It was also called as 'break-heart fever' and 'la-dengue'. 'infectious thrombocytopenic purpura' and 'Philippine', 'Thai or Singapore haemorrhagic fever' these terms were used for severe disease (Halstead, 2008)

Another meaning of dengue is 'affected' to describe the outbreaks reported in 1635 in the Caribbean and 1699 in Central (Murray et al., 2013b). Before World War II (WWII), as the urban centers in the tropics were relatively small and ocean going ships were the major means of international transportation, dengue epidemics were minimized and each region had predominant circulating virus strains. Thus dengue carried little public health interest (Gubler, 2011). History recorded epidemics that occurred periodically in coastal cities throughout the tropics and subtropics. During 17^{th} and 18^{th} centuries dengue fever spread to tropical cities worldwide, likely due to expansion of *Aedes aegypti* from its homeland Africa to Asia and Americas (Powell and Tabachnick, 2013). During WWII, dengue spread most of the remote regions of Southeast Asia and the pacific Islands due to massive movement of soldiers. In Pacific region, at that time dengue was considered as one of the major two threats (another one was malaria) especially for US military that accounted for 20-30% undifferentiated fever in Vietnam, Somalia and Haiti (Walter Reed Army Institute of Research). By the end of WWII, many Asian countries became hyper endemic with all 4 strains (Gubler, 2011). After WWII, South East Asia (SEA) had become most affluent DHF epidemic zone due to dramatic expand of globalization and urbanization. In 1953-54, the first such epidemic was reported in Philippines followed by Thailand in 1958, then Malaysia, Singapore, Vietnam, Indonesia and Myanmar in 1960s and 1970s. By the 1980s, DHF was a major threat for childhood illness and

mortality in some SEA countries (Gubler, 2011). Following WWII, though Latin America had same experience of population growth and urbanization epidemics were cut short through major public health campaign. Pan American Health Organization (PAHO) arranged a broad scale international campaign with a view to eliminate the *Aedes* from the hemisphere. Although the main target of the campaign was to eliminate Yellow Fever; dengue rates declined concomitantly as the two viruses share same vector. Majority of Brazil and other South American nations succeed to eliminate the mosquito. By 1947, continental *Aegypti* eradication plan was adopted with the wide spread use of DDT insecticide. Unfortunately, early success was not met. As a result, in 1960s dengue outbreak occurred and reintroduction of an Asian dengue virus, DEN3 to Caribbean was noted (Dick et al., 2012).

In Indian subcontinent, after 1990s, DF and DHF cases were increasing and outbreaks were recorded in all countries with India, Pakistan, Bangladesh and Sri Lanka on the fore front (Raheel et al., 2011). Though other SEA countries Bangladesh is a suitable habitat for mosquito; only sporadic cases were reported from Dhaka (capital of the country) and other parts of the country (Russell et al., 1966; Amin et al., 2000). A sudden outbreak occurred in 2000, when 5,551 cases and 93 deaths were reported. From 2000-2017, during outbreaks both *aegypti* and *albopictus* were found habituated in Bangladesh. Like other Lower and Middle Income Countries (LMICs) it was expected that the virus would remain in the environment since the vector might have been always present and would have caused serious outbreaks in near future (Mutsuddy et al., 2019). Finally, in 2019 the apprehension came true as the country faced the worst dengue attack that twitched entire health system of the country. According to WHO, not only in Bangladesh high number of cases were reported also in Malaysia (1,31,000), Philippines (4,20,000), Vietnam (3,20,000) in Asia and transmission of dengue virus was recorded in Afghanistan for the first time. It was the most devastating dengue outbreak in the global history and its vitality is still ongoing in 2020 (WHO, 2020).

2.2: Landscape of 2019 dengue outbreak in Bangladesh

It is estimated that around 75% of world's dengue burden is in Asia, especially in countries such as the Philippines, Indonesia and Thailand (Wilder-Smith et al., 2019). However, the situation changed when South Asian (SA) countries, Bangladesh had the largest dengue outbreak. Bangladesh once was considered as a lower sero-

prevalent country than many other countries in the SEA (Sharmin et al., 2015). The total patients with death reported between 2000 and 2018 shown in the Table 1 below (Hasan, 2019).

Table 1: DENGUE IN TWO DECADES

YEAR	ADMISSION	DEATH	YEAR	ADMISSION	DEATH
2000	5,551	93	2011	1,359	6
2001	2,430	44	2012	671	1
2002	6,232	58	2013	1,749	2
2003	486	10	2014	375	0
2004	3,434	13	2015	3,162	6
2005	1,048	4	2016	6,060	14
2006	2,200	11	2017	2,769	8
2007	466	0	2018	10,148	26
2008	1,153	0	*Total	50,176	296
2009	474	0	2019 (till Oct 13)	91,866	93
2010	409	0	Grand Total	1,42,042	389

Note: Number of dengue patient admissions in 2019so far is almost double (1.8 times) the total figure from 2000-2018.

Up to 2018, according to DGHS, the highest recorded number of deaths from dengue was 93 in 2000 and total 296 people died from 2000 to the end of 2018. In contrast, Epidemiology Disease Control and Research (IEDCR) announced that 93 dengue patients died between 1st January and 13th October this year alone. According to DGHS, numbers of dengue-infected patients were 38 in January, 18 in February, 17 in March, 58 in April, 193 in May, 1884 in June, 16,253 in July, climaxing in August to 52,636. The figure then started lowering with 16,856 in September, 8,143 in October, 4,011 in November and 1,237 in December. Although after the monsoon the number of dengue cases usually peaks in October but this year surge started from May; mostly due to substantial increase in rain fall; creating a breeding condition for *Aedes* mosquitoes (Maswood, 2020). On an account, last year 101,354 people were admitted into hospital with dengue throughout the country and the matter of relief

that, of them 101,037 got full recovery. The government of the country claimed that total 164 people died from dengue and IEDCR confirmed the number after reviewing 263 out of 266 reports of dengue related deaths (DGHS, 2020). However, according to experts, the actual number of the cases could be several times greater than officially reported due to poor surveillance system. The reports of cases and deaths by the disease were to send to the DGHS by 12 government and 29 private hospitals in the capital and by 64 civil surgeons and medical college hospitals elsewhere in the country. Critical patients had to be referred to the capital as the district health facilities were not adequate for the situation and many of the patients died on the way. As a result, the actual number of cases and deaths remained controversial (Hasan, 2019).

2.3: Dengue vector

Like other SEA countries Bangladesh is situated in the tropical and subtropical regions and has become a suitable habitat for the dengue vector and its increased transmission (Mutsuddy et al., 2019). Precisely, the havoc wreaking dengue virus is transmitted by its notorious carrier: the Aedes mosquito. According to professor Jeffrey R Powell of Yale University, Aedes mosquito is the most dangerous animal in the world in his paper titled ‘new contender for most lethal animal (Gulshan, 2019). Mosquitoes constitute the large family Culcidae that consists of 2 subfamilies: Anophelinae (3 genera) and Culicinae (110 genera). *Aedes* is the largest tribe of Culicinae with 1256 species (Kweka et al., 2018). There are several other Aedes species apart from *A. aegypti* and *A. albopictus*; such as: *Aedes polynesiensis* and several other species of *Aedes scutellaris* complex that can transmit diseases to humans and in many instances any of several different species can transmit the same pathogen. Different species have different particular ecology, behavior and geographical distribution (WHO, 2019). The widespread *aedes* mosquito has both domestic populations as well as the ancestral type that still extent in Sub-Saharan Africa (Powell and Tabachnick, 2013).

The life cycle of mosquitoes, regardless of species or genus consists of 4 distinct stages: eggs, larvae, pupae and adults requiring 2 essential elements: vertebrate blood and other is water. The water is necessary for providing the physical

environment for the overall development from egg to adult through pupa and larva and the blood is necessary for making eggs. Study says that, Aedes mosquito was originally adapted to forest habitat wherein it used to lay eggs in holes in trees that would regularly collect rainwater. However, as humans destroyed this forest habitat for establishing agriculture; Aedes mosquito readily adapted to the new circumstances. 'The mosquitoes found an abundance of new and highly effective small containers strewn in and around households that can easily collect, or are intended to store water.' Instead of, in the water or on the surface of the water (as most species do) Aedes mosquito lays its eggs above the water on the interior wall of the container. As a result, when the container is refilled with water; from the water line at, which mosquito laid its egg the lip of the container; the eggs will get enough time to complete their developmental cycle to adulthood before evaporation depletes the water source. Thus, Aedes mosquito is now considered as an urban mosquito rather than a forest mosquito as it is now uniquely and evolutionary adapted to the humans environment. Even, this species can often live in the households with humans and can complete its whole life-cycle here (Walsh, 2011). However, *Aedes albopictus* tended to occur more commonly in areas with open spaces and vegetation (WHO, 2011)**.**

The *Aedes* prefers to bite during the day to get unlimited access to humans. During daylight, for approximately 2 hours after sunrise and several hours before sunset the mosquito is most active although peaks of biting activity may vary with location and season (Lumsden, 1957; Nelson et al., 1978). In indoors, the mosquito rests in closets and other dark, humid places and at outside, less often, it is found in vegetation or other protected sites. As they reside in non-sprayable surfaces indoor residual spray is not an option for its control as with malaria vectors (WHO, 2011). It takes about 3-4 weeks to complete the life cycle of Aedes. During a lifetime, the females can produce on an average 50-120 egg in each batch in small containers. Development within the eggs is faster in warm climates such as little as 2 days, whereas it may take longer time in cooler temperate climates. Once the embryonic development is completed, these can survive for a very long period in a dry state, even for more than a year. The eggs hatch immediately once submerged in water. All eggs do not hatch at the same time (WHO, 2011).

Larval development also depends on temperature and availability of food and larval density. When the temperature is cool, larval stage can continue for several weeks so long as the water supply is sufficient and under optimal conditions. The time taken from hatching to the emergence of the adult can be approximately 10 days and as short as seven days, including two days in the pupal stage (WHO, 2011).

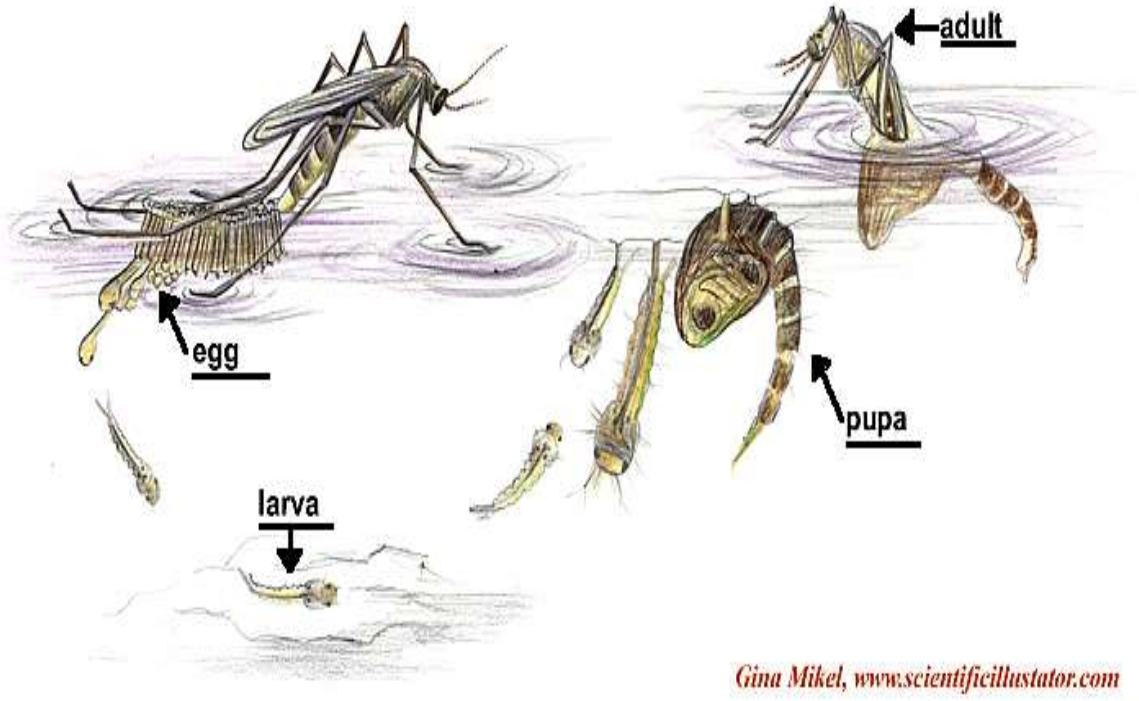

Figure 1: Life cycle of Aedes mosquito.

Adult Aedes has head, neck, thorax and abdomen like mouth brush, spines, compound eye, antenna, comb spines, siphon tube, pectin teeth and anal papilla. Males are relatively smaller than the females in size. Males do not bite humans or animals and their mouthparts are modified for nectar feeding. On the other hand, mouthparts of female mosquitoes are modified for blood feeding (Fahad et al., 2018). These female mosquitoes bite so innocuously usually on the back of the neck and the ankles that they are easily disturbed during blood meal. This causes them to move on to finish a meal on another individual, making them efficient vectors (Smith., 2019).

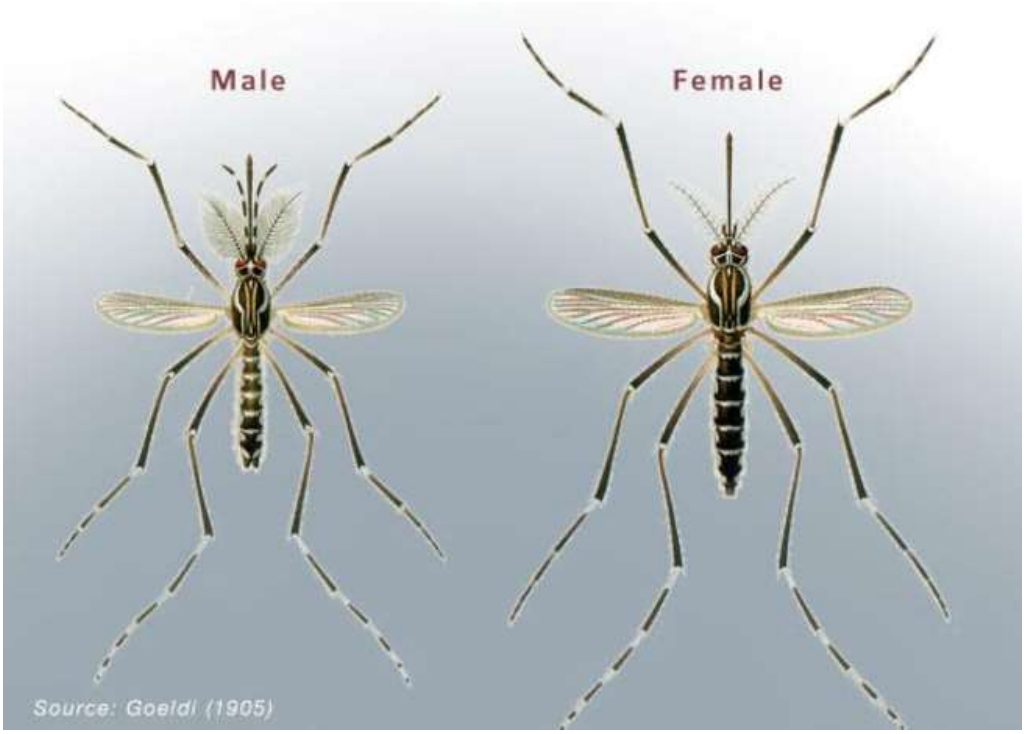

Figure 2: Aedes mosquito

2.4: Virology of Dengue virus

'The virus responsible for causing dengue, is called dengue virus (DENV)' (WHO, 2014). It is a single positive stranded RNA virus; Family: Flaviviridae and Genus: Flavivirus (Rodenhuis-Zybert et al., 2010). The genus Flavivirus also includes the West-Nile virus, Tick-borne encephalitis virus, Yellow fever virus, Zika virus and several other viruses those can cause encephalitis (Shi, 2012). About 11000 bases of positive-sense single stranded RNA (ss RNA) of DENV code for 3 structural proteins and 7 nonstructural proteins. Three structural proteins are Capsid protein C, Membrane protein M, Envelope protein E and7 nonstructural proteins are NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. The genome RNA also includes short non-coding regions on both the 5' and 3' ends (Hanley and Weaver, 2010; Rodenhuis-Zybert et al., 2010). While Envelope, Membrane and Capsid proteins form the viral particle, the nonstructural (NS) proteins assemble inside a cellular replication complex, which embedded in endoplasmic reticulum (ER) derived vesicle. NS proteins also help the virus to escape the host innate immunity and reshape the host cell inner structure (El Sahili and Lescar, 2017). Studies revealed that, NS1 protein is the only protein that secreted continuously by infected host cells and it has been used as an early diagnostic marker for dengue infection for many years. Besides, NS1 protein may play a role in

vascular leakage, coagulopathy and thrombocytopenia during dengue infection (Chen et al., 2018).

2.4.1: Virus Structure and Composition

After Yellow fever, Dengue fever was the 2nd human disease whose etiology was critically identified as a 'filterable virus' (7,238) in 1970 it was Ashburn and Craig who provided the first data demonstrating the filterable, ultramicroscopic character of etiological agent (Ashburn and Caraig, 1907).

2.4.1 (a): Physiochemical properties of the virion

Mature dengue virions have a single stranded RNA genome surrounded by an approximately icosahedral or isometric nucleocapsid about 30 nm in diameter. A lipid envelope of about 10 nm in depth covers the nucleocapsid. A complete virionis about 50 nm in diameter (Russell et al., 1980).

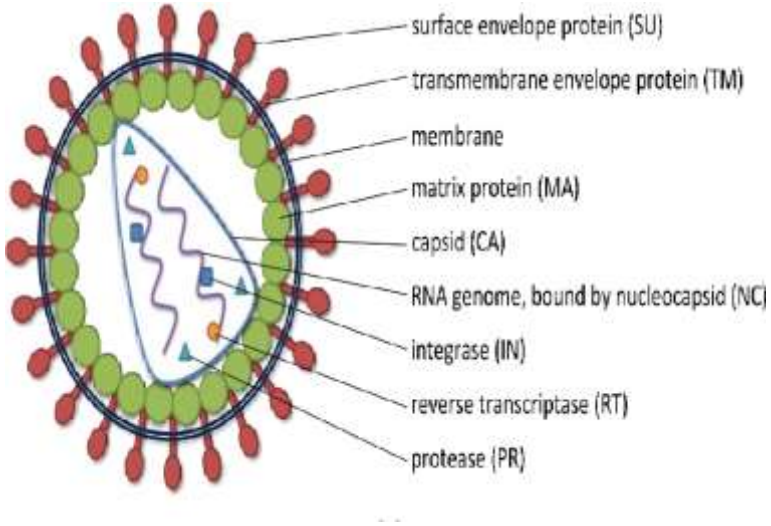

Figure 3: Structure of Dengue Virus (Oviya et al., 2019).

2.4.1 (b): The viral genome:

Like other Flavi viruses, the molecular weight of single stranded RNA of Dengue virus is around 3.3×10^6 (Stollar et al., 1966). This RNA is infectious, has a molecular like positive polarity (Svitkin et al., 1984). This RNA lacks a poly (A) tail at the 3′ end (Irie et al., 1989) and the 5′- end is capped (Wengler et al., 1978). It has been observed that, though initiation and translation process takes place at 5′end and RNA replication initiates at 3′- end of the genome, at the 3′- end cis-acting elements

required for translation, initiation can also be found. Besides, necessary elements for initiation of RNA synthesis can also be found at 5′- end of the viral RNA (Filomatori et al., 2006). So the RNA acts as promoters, enhancers and repressors of translation, transcription, replication and encapsidation (Iglesias and Gamarnik, 2011). Also, viral RNA can participate in triggering or avoiding the antiviral host response (Baum et al., 2010; Daffis et al., 2010).

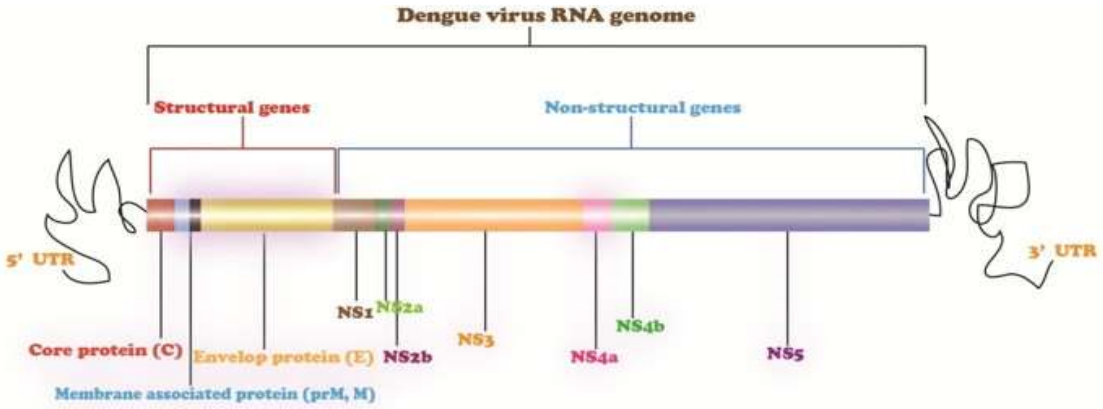

Figure 4: Dengue viral genome, structural and nonstructural proteins (Idrees and Ashfaq, 2012)

2.4.1 (c): Viral structural proteins

Rice and co-workers first gave the currently accepted nomenclature for flavivirus structural and nonstructural proteins. A mature virion possesses 3 structural proteins: C, the nucleocapsid or core protein; M, a membrane associated protein; and E, the envelope protein (previously stated). Besides, an immature one mainly the intracellular virus contains a protein known as prM (sometimes preM), a precursor of M (Shapiro et al., 1972). Genes responsible for encoding the structural proteins are located at the 5′ end of the RNA (Zhao et al., 1986; Osatomi et al., 1988) from the 5′ end, the gene order for the structural proteins is C-prM(M)-E. The precursors for the proteins is a single, long, precursor polypeptide or polyprotein (Zhao et al., 1986; Mason et al., 1987; Osatomi et al., 1988). The molecular weight of the C protein is about 13,500 dalton. This highly basic protein is the first viral polypeptide synthesized during translation (Zhao et al., 1986; Mason et al., 1987; Osatomi et al., 1988). It is synthesized on non-membrane bound ribosomes as it lacks N-terminal, hydrophobic signal sequence. As a result, a hydrophobic stretch of amino acids at the C protein carboxy terminus acts as a ‘transmembrane signal’ for the adjacent M protein precursor; prM and thus the C protein becomes anchored transiently to a

membrane at the replication site after cleavage (Markoff, 1989; Nowak et al., 1989). Before the completion of virus maturation, a virus encoded protease cleave the hydrophobic domain from C protein (Nowak et al., 1989).

The mature DENV glycoprotein shell consists of 180 copies of each of the E and M proteins. These two proteins are closely associated until the viral particle is released into extracellular environment (Perera and Kuhn, 2008). The molecular weight of M protein is 8,000 dalton. This protein is resulted from a proteolytic cleavage of glycosylated prM precursor (22,000 dalton) during virus maturation (Mason et al., 1987; Hahn et al., 1988; Osatomi et al., 1988). This cleavage occurs in the acidic post Golgi vesicles, appears to precede viral release from the cell. Therefore, the formation of M protein from prM is very important and terminal event in virion morphogenesis and recognition of virus surface structure. Immature virions are highly stable and relatively inert whereas mature one is more competent for infection (60 folds) but more labile (Wengler, 1989; Randolph et al., 1990). It is E-prM heterodimers which composes the immature viral surface structure (Wengler, 1989) while the major virion glycoprotein is the E glycoprotein (51,000 to 60,000 dalton) that covers the surface of mature virions as homotrimer (Wengler et al., 1987). At first, Nowak and Wengler provided a structural model for the envelope protein (Nowak and Wengler, 1987)which was further refined by Mandl et al (Mandl et al., 1989). The recent model of Flaviviral E protein is based on 3 non overlapping antigenic domains (composed of at least 16 distinct epitopes): A, B and C with disulfide bridge assignments. The integrity of domains A and B, which is composed of discontinuous epitopes, depends on intact disulfide bridges. On the other hand, the highly variable domain C lacks disulfide bridges. As a result, reduction, Carboxymethylation, or Sodium dodecyl Sulfate denaturation can't destroy the epitopes in this region (Nowak and Wengler, 1987; Mandl et al., 1989).

2.4.1 (d): Composition and function of Dengue Viral Envelope

In 1980, Russell and co-workers last reviewed the lipid composition of virion envelope (Russell et al., 1980). The lipid composition is with minor exceptions, similar with that of the host cell membrane (probably, intracellular membranes of Endoplasmic Reticulum (ER) (Boulton and Westaway, 1976; Brinton, 1986). An

important function of this lipid envelope is to protect nucleocapsid from RNase. During penetration and un-coating it fuses with the host cell membrane, presumably promoted by a unique fusion domain of E protein (Gollins and Porterfield, 1986; Kimura et al., 1986).

2.4.1 (e): Virus non-structural proteins

Seven, non-overlapping, nonstructural (NS) proteins are encoded 3' to the structural protein-coding region and following the E protein are NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5. The first nonstructural protein, NS1 (≈48,000 MW) is a glycoprotein that arises in viral morphogenesis (Rice et al., 1986). The NS2 coding region codes for 2 proteins: NS2a (≈20,000 MW) and NS2b (≈14,500). NS2a is necessary for proteolytic processing of C-terminus of NS1 (74). The function of NS2b is not known in virus replication (Speight et al., 1988; Chambers et al., 1989). The NS3 (≈70,000 MW) is a hydrophilic protein which may act as a protease. Recently, NS4a (≈16,000 MW) and NS4b (≈27,000 MW) have been figured at in the viral RNA and may act as replication complex co-factors along with NS5. NS5 (≈105,000 MW) is large thought to be an RNA dependent RNA polymerase (Rice et al., 1986; Grun and Brinton, 1987; Sumiyoshi et al., 1987).

2.4.2: Dengue virus replication scheme

2.4.2 (a): Attachment, penetration and un-coating

Attachment of dengue virus to susceptible cells occurs by one of the two mechanisms:

1) Dengue virus makes complex with non-neutralizing anti-virion IgG and this complex attaches to Fc receptors on the surface of Macrophages (MQ) or monocytes ('Immune infection enhancement' (Daughaday et al., 1981; Grun and Brinton, 1987).

2) It may attach to a trypsin-sensitive virus receptor on the surface of the cells including monocytes (Daughaday et al., 1981).

Penetration of the attached infectious virus to the host cells also occurs via one of the two methods:

1) Fusion of the virion envelope with the plasma membrane and immediate deposition of the nucleocapsid into the cytoplasm.

2) Invagination of plasma membrane around the enveloped virus forming an endocytic vesicle (endosome).

Electron microscopic study showed that, penetration of dengue virus (also, Japanese Encephalities virus) to the plasma membrane and/or macropinocytic vacular membranes of mosquito cells and/or human peripheral blood monocytes occurs through membrane disruption at adsorption sites (Hase et al., 1989).

2.4.2 (b): Primary translation and early RNA replication

Very early mechanisms in dengue viral replication are not known. As the viral RNA genome has a positive sense, first it undergoes translation process to make the RNA polymerase required for its replication. The enzyme then transcribes the positive-strand RNA to negative strand RNA, which then serves as a template for additional positive strands (Henchal and Putnak, 1990).

2.4.2 (c): Synthesis and proteolytic processing of viral proteins

At the site of the first AUG codon, the translation process begins (Rice et al., 1985; Zhao et al., 1986; Mason et al., 1987; Hahn et al., 1988). The individual viral proteins are formed by co-translational proteolytic processing of the precursor or peptide which is a large polyprotein encoded by a long, open reading frame (Rice et al., 1985).

2.4.2 (d): RNA replication

RNA replication can be detected as early as 3 hours post infection occurring in the perinuclear region of the infected cell in association with smooth membranes (Takeda et al., 1978). Unfortunately it is unknown how the replication of a full length, double stranded RNA containing one positive strand completely annealed to one negative strand is regulated (Cleaves et al., 1981; Chu and Westaway, 1985). However, late replication process involves the synthesis of infectious positive strand (Stollar et al., 1967; Westaway, 1980). Assembly of the nucleocapsids and removal of positive strands as a substrate for replication favored by the increasing concentration of C protein late in infection. The continued translation and reason behind the predominance of positive strand RNA later in infection is also explained by C protein as it binds to a site at the 3′ end of positive strand RNA that prevents it from being recognized by RNA polymerase but not by Ribosomes which bind at 5′end.

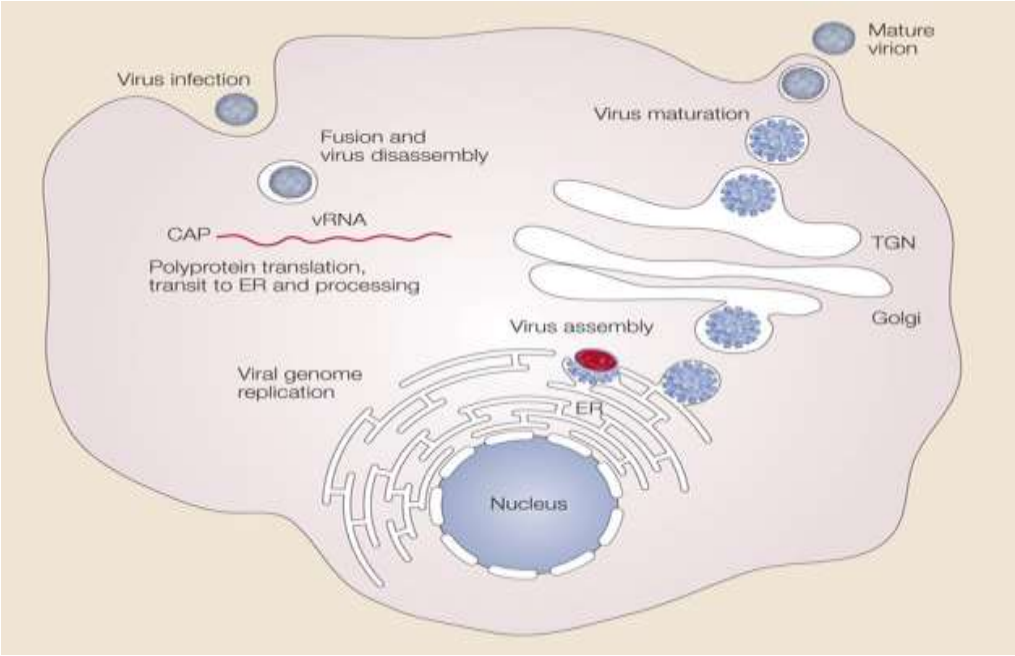

Figure 5: Dengue virus replication (Bisimwa, 2015)

After the attachment to the surface of a host cell the virus enters the cell by a process called endocytosis. Once deep inside the cell, fusing with the endosomal membrane the virus particle comes apart, releasing the viral genome which is then translated into a single polypeptide that cut into ten proteins, and the viral genome is replicated. The structural proteins and newly synthesized RNA bud out from the ER. Trans-Golgi network (TGN) transports the immature viral particles where they mature and convert to their infectious form. The mature viruses then get release from the cell and continue to infect other cells (Bisimwa, 2015).

2.4.2 (e): Virus assembly and release

Assembly of the viruses occurs through 4 phases:

1) Nucleocapsids assemble from C protein and RNA;

2) Nucleocapsids bud through membrane containing integral E and prM proteins to acquire an envelope;

3) Get exit from the cell, either as a result of the budding process or, afterwards, in exocytic vesicles, and

4) prM protein is cleaved that results in a reorganization of the virion surface and virion maturation (Henchal and Putnak, 1990). Finally, progeny viruses release from the infected cell presumably via secretory exocytosis as virus-containing secretory vesicles fuse with the plasma membrane (Leary and Blair, 1980; Hase et al., 1987).

2.4.3: Dengue serotypes

Dengue virus (DEN) comprises of 4 epidemiologically related but antigenically distinct 4 serotypes (DEN-1, DEN-2, DEN-3, DEN-4) (Endy et al., 2002b), which can co-circulate and have capacity to produce severe dengue disease, demonstrated as early as 1960 (Halstead et al., 1969). During WW 2 DEN-1 and DEN-2 were first isolated from US soldiers and in 1954 DEN-3 and DEN-4 were isolated from patients with dengue hemorrhagic fever in the Philippines and Thailand (Sabin, 1952; Hammon et al., 1960). Four serotypes show extensive genetic variability as within each serotype there are several clusters of variants termed as genotypes. These genotypes vary by ≈6% and 3% at the nucleotide and amino acid levels respectively. In addition, the 4 serotypes differ by ≈25-40% at the amino acid levels (Holmes, 2009; Rico-Hesse, 2010). Scientists observed that, different dengue serotypes and even strains of the same serotypes differ in their tendency to cause severe disease (Nisalak et al., 2003; Rico-Hesse, 2003). Although, there is no clear evidence on the association of DHF and severe disease with serotype. Analyses of data collected during various DF and DHF outbreaks worldwide have shown association of DENV2 (Russell et al., 1968; Halstead, 1980; Burke et al., 1988; Thein et al., 1997) and DENV3 (Endy et al., 2002a; Nisalak et al., 2003) with increased risk of hospitalization and severe disease. Genetic studies of sylvatic strains suggested that, approximately 1000 years ago, the 4 serotypes evolved from a common ancestor in primate populations. 500 years ago, they separately emerged into a human urban transmission cycle in either Asia or Africa (Wang et al., 2000; Kyle and Harris, 2008). However, a 5^{th} serotype was reported in 2013 (Normile, 2013). All variants follow human cycle except the new variant DENV5 that follows the sylvatic cycle that lead the possibility of existence of additional sylvatic dengue strains that can further impede the Dengue Vaccine Initiative (Mustafa et al., 2015).

Table 2: Dengue serotypes Status in Different Years (Akram, 2019)

Year	Dengue serotypes Status
2013 to 2016	DEN2 (predominant) followed by DEN1
2017	DEN 2 (predominant) followed by DEN1 and co detection of DEN 3 with DEN 2 (few cases)
2018	DEN 2 (predominant) followed by DEN2 and DEN1 and co-detection DEN2 and DEN3 and DEN1 and DEN3(few cases)
2019	DEN 3 (predominant) followed by co-detection of DEN2 and DEN3 and DEN1 and DEN3 (few cases)

These viruses have been characterized as having a low, medium, or high epidemiological significance according to their likelihood for human transmission and clinical severity of dengue epidemics. Some viruses largely maintain sylvatic cycles; rarely transmitting the disease to humans or causing mild dengue fever while other genotypes having higher virulence in relation with cases and epidemics characterized by more severe disease manifestation (Rico-Hesse, 2003). For example, some DENV2 and DENV3 genotypes found commonly in the Americas, which are comparatively less virulent than Asian genotypes of the same serotype (Rico-Hesse, 2003; Wilder-Smith et al., 2010). As the dengue viruses are spreading throughout the world, phylogenetic and epidemiological studies revealed that the genotypes that are more virulent are now displacing those that have lower epidemiological impact and there is no evidence for the transmission of antigenically aberrant, new strains (Rico-Hesse, 2003). Based on clinical, biological and immunological criteria, it is recognized that dengue viruses belong to a distinct virus complex and there is existence of complex-specific antigenic determinants (Smithburn, 1954) on the NSI protein (Russell et al., 1970). Later on, by using mouse monoclonal antibodies, specifically identified the complex reactive epitopes on structural and nonstructural antigens of Flaviviruses and in 1950, Sabin demonstrated that, for the presence of these epitopes, human anti-dengue convalescent sera would cross react with other flaviviral antigens (Sabin, 1950; Henchal and Putnak, 1990). So, though life-long immunity can be achieved after infection with one dengue serotype, temporary (or no) cross protective immunity to the other serotypes will be gained due to formation of

heterotypic, anamnestic response (Levinson, 2016). Residents of an endemic region can be infected with more than one serotypes concurrently and sequentially (Reddy et al., 2017).

2.4.4: Dengue virus transmission

Potential local transmission established when there is presence of susceptible vectors in a certain area and infected persons would travel that area. 2 usual ways of Dengue virus transmission are:

1) Mosquito-to-human transmission: Dengue virus is transmitted to humans by the bite of infected female Aedes mosquitoes, primarily *Aedes aegypti* (stated before). When a female mosquito bites an infected person, the virus starts replicating itself in the mosquito mid gut before it disseminates to secondary tissues, including salivary glands (stated before). The period from the ingestion of the virus to the actual transmission to a new host is called the Extrinsic Incubation Period (EIP) which is about 8-12 days when the ambient temperature is between 25-28ºC (Siler et al., 1926; Watts et al., 1987; Tjaden et al., 2013). EIP depends not only on the ambient temperature but also on the magnitude of daily temperature fluctuations (Lambrechts et al., 2011; Carrington et al., 2013), virus genotype (Anderson and Rico-Hesse, 2006) and initial viral concentration (Ye et al., 2015).

2) Human-to-mosquito transmission: Most of the humans remain viremic for about 4-5 days, but viremia can last as long as 12 days (Gubler et al., 1981). Mosquitoes become infected from the people who are viremic with DENV. Symptomatic, pre-symptomatic, and even asymptomatic person can be a source of infection (Duong et al., 2015). Infection can be transmitted up to 2 days before someone shows symptoms of the illness (Siler et al., 1926; Duong et al., 2015), up to 2 days after the fever has resolved (Nguyet et al., 2013).

Unusual modes of transmission: there are some rare case reports that documented dengue virus transmission without a mosquito vector. The rare routes can be needle stick injuries, blood transfusion, bone marrow transplantation, intra partum and vertical transmission and so on (Chen and Wilson, 2004). A case of dengue transmission from a donor to a recipient after liver transplantation (Gupta et al., 2016). A case of vertical transmission through breast milk was also reported (Barthel et al., 2013).

2.5: Epidemiology of Dengue

Over the last half-century, dengue virus and its vectors became so widely distributed throughout tropical and subtropical regions of the world that with rapid increases in incident of cases, epidemics and hyperendemicity would lead to more severe forms of dengue (Murray et al., 2013a). As early as 1635 and 1699 in West Indies and Central America respectively epidemics occurred that resembled dengue with similar disease course and way of spread (Gubler, 1997). Finally, in 20th century, it was possible to establish the viral etiology and the mode of transmission by mosquito vectors. With the pace of time, especially after WW2; commercial expansion, rapid urbanization and modern transportation led to increased transmission of dengue and hyperendemicity in most SE Asian countries with subsequent emergence of the severe forms of dengue (Gubler and Clark, 1995; Wilder-Smith and Gubler, 2008; Gubler, 2011). Matter of fact that, we still know little about the dengue, however; what has become important to know over the years of research is that, dengue epidemiology is determined by many factors, including those in host, vector and environment creating a very complex interaction (Rico-Hesse, 2003). Along with the specific viral structure and increased transmission by the mosquito vector; the immune status and possibly the genetic background of the host are also determinants of virulence or the disease presentation (Rico-Hesse, 2003). As well as, high temperature, humidity and precipitation, heavy rainfall associated with climate change show possible increase in dengue transmission (Hales et al., 2002; De Souza et al., 2010). The children were considered as higher vulnerable group in previous case reports but with the changing trend; adult infection is increasing in certain countries as the most travelers with dengue have been adults (Wilder-Smith and Schwartz, 2005; Ooi et al., 2006; Cummings et al., 2009). The actual disease burden substantially remains underestimated as most of the cases are asymptomatic, and persons with mild illness may not seek medical attention (Ferreira, 2012).

It causes a significant health, economic and social burden globally that the WHO has classified Dengue as a major international public health concern (Wilder-Smith et al., 2010). It was hypothesized that in near future it would further increase in geographic expansion, incidence and reporting to WHO (Hales et al., 2002; Wilder-Smith and Gubler, 2008; Wilder-Smith et al., 2010; Åström et al., 2012). There is still lack of uniformity in dengue case definitions, in diagnosis ascertainment, in

laboratory capacity and the diversity of public health practices in each country. Also lack of sustainable prevention or control measures along with absence of an effective vaccine; only focus on the early identification and early response to outbreaks can merely do little in reality and thus shaping Dengue more unpredictable (Ferreira, 2012). 'In summary, the global trends of dengue disease epidemiology are characterized by a rapidly expanding geographic distribution of vector infestation, the risk of infection, and disease transmission, despite ongoing control efforts. An increased frequency and magnitude of epidemics with significant levels of hospitalization and a marked increased risk for severe forms of dengue are associated with the continual circulation of the four-dengue virus serotypes. Dengue is therefore an increasingly global public health concern, characterized by unpredictable epidemics, and for which no sustainable control measures currently exist'(Ferreira, 2012).

2.6: Clinical course

Early detection and understanding of clinical problems during the different phases of dengue is very much important for proper management and good clinical outcome. The wide spectrum of dengue consists of both severe and non-severe clinical manifestations. After the incubation period of 5-7 days, it begins abruptly and is followed by 3 phases: febrile, critical and recovery (WHO, 2009).

1) Febrile phase: it starts with sudden onset of high-grade fever often associated with facial flushing, skin erythema, generalized body ache, myalgia, arthralgia and headache (Rigau-Pérez et al., 1998). There may be sore throat, injected pharynx and conjunctival injection. Anorexia, nausea and vomiting are very much common as other viral fever. A progressive decrease in total White Blood Cell (WBC) count is the earliest abnormality. This acute febrile phase lasts for 2-7 days and may progress to critical phase if adequate monitoring for warning signs is not taken as the clinical features are very much indistinguishable between severe and non-severe dengue cases (WHO, 2009).

2) Critical phase: this phase usually begins on days 3-7 of illness (time of defervescence) with a drop of temperature to 37.5-38ºC or less and remains below this level as well as an increase in capillary permeability in parallel with increasing haematocrit levels (Nimmannitya et al., 1969; Srikiatkhachorn et al., 2007). There is

progressive decrease in WBC count (Kalayanarooj et al., 1997) along with rapid decrease in platelet count that usually precedes plasma leakage. Clinically significant plasma leakage period usually lasts for 24-48 hours. When there is increased capillary permeability, shock precipitates due to loss of critical volume of plasma through the leakage. As a result, organ hypo perfusion leads to progressive organ impairment, metabolic acidosis and disseminated intravascular coagulation (DIC). In severe shock, this condition further leads to severe haemorrhage causing the haematocrit to decrease. Instead of leukopenia, leukocytosis may be seen in patients with severe bleeding (Martinez-Torres et al., 2008). However, severe organ impairment and/or severe bleeding may also occur without obvious plasma leakage or shock (Martinez-Torres et al., 2008). After defervescence, non-severe dengue cases recover. Even, without defervescence some patients may progress to critical phase of plasma leakage. There are some warning signs that indicate that patient is going to be a critical case (WHO, 2009).

Table 3: Warning signs (WHO, 2012)

Clinical	Abdominal pain or tenderness Persistent vomiting Clinical fluid accumulation Mucosal bleeding Lethargy, restlessness Liver enlargement > 2 cm
Laboratory	Increase in haematocrit concurrent with rapid decrease in platelet count.

3) Recovery phase: After critical phase, recovery occurs in 48-72 hours. General condition of the patient improves, appetite returns, gastrointestinal symptoms subside, haemodynamic stasus stabilizes and diuresis ensues. The haematocrit level rises or may be lower, WBC count starts to rise usually after defervescence but the platelet count recovers in a slow pace than that of WBC (WHO, 2009).

Table 4: Febrile, critical and recovery phases in dengue (WHO, 2012)

Febrile phase	Dehydration; high fever may cause neurological disturbances and febrile seizures in young children.
Critical phase	Shock from plasma leakage; severe haemorrhage; organ impairment.
Recovery phase	Hypervolaemia (only if intravenous fluid therapy has been excessive and/or has extended into this period)

2.7: Clinical manifestation of dengue

Dengue takes a typical incubation period of 5-7 days to begin abruptly. The clinical spectrum of dengue viral infections extents from asymptomatic to severe illness that may lead to death if not properly managed (Kalayanarooj, 2011). It is estimated that 1 in 4 patients become symptomatic in this infection (CDC, 2019a). The symptomatic cases are classified as: undifferentiated febrile illness (UF), dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS) and unusual dengue (UD) or expanded dengue syndrome (EDS) (WHO, 2011).

1) Undifferentiated febrile illness (UF): person clinically has no fever and the diagnosis is only based on serology or virology (Kalayanarooj, 2011).

2) Dengue fever (DF): clinical manifestations of DF frequently depend on the age of the patients. An undifferentiated febrile disease may have seen in infants and younger children with or without a maculo-papular rash. On the other hand, older children and adults may suffer from either a mild febrile syndrome or the classic incapacitating disease with high fever of abrupt onset, sometimes with 2 peaks (saddle-backed), severe headache, pain behind the eyes, muscle and bone or joint pains, nausea and vomiting, and rash. Skin haemorrhages (petechiae) also may be seen. Laboratory blood picture commonly shows leukopenia; thrombocytopenia may be observed. In adults, recovery may be accompanied by prolonged fatigue and depression. DF with bleeding complications, such as epistaxis, gingival bleeding, gastrointestinal bleeding, haematuria, and menorrhagia also may be seen in some epidemics (WHO, 1997).

3) Dengue haemorrhagic fever (DHF): increase in vascular permeability (plasma leakage) is the differentiating point between DHF and DF; otherwise, clinical

presentations in febrile phase are almost similar to those in DF (Kalayanarooj, 2011). In addition, plasma leakage determines the severity of DHF and it manifested by increased haematocrit, a serous effusion or hypoproteinaemia. However, 4 major typical features of DHF are: high fever, haemorrhagic phenomena, and often, hepatomegaly and circulatory failure. Laboratory blood picture distinctively shows moderate to marked thrombocytopenia with concurrent haemoconentration (WHO, 1997). Infants and children mostly suffer from DHF (Sellahewa, 2012). It starts with sudden rise of usually high temperature (>39º C) accompanied by facial flush and other non-specific constitutional symptoms. Temperature usually remains so for 2-7 days and occasionally, it is as high as 40-41ºC that febrile convulsion may occur, especially in infants. Positive tourniquet test that is easy bruising and bleeding at venipuncture sites is the most common haemorrhagic phenomenon in DHF. Tender hepatomegaly is more frequent in cases with shock than cases with non-shock. The critical phase starts after 2-7 days of onset of fever when temperature rapidly goes down accompanied by signs of circulatory disturbance of varying severity that profound shock and death may ensue if not treated promptly.

Table 5: Grading of the severity of DHF (WHO, 2011)

Grade 1	Fever accompanied by nonspecific constitutional symptoms; the only haemorrhagic manifestation is a positive tourniquet test.
Grade 2	Spontaneous bleeding in addition to the manifestations of Grade 1 patients, usually in the form of skin and/or other haemorrhages.
Grade 3	Circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mmHg or less) or hypotension, with the presence of cold clammy skin and restlessness
Grade 4	Profound shock with undetectable blood pressure and pulse.

3) Dengue shock syndrome: between the 3rd and 7th day of the disease the condition of the patients who progress to shock suddenly deteriorates. There is rapid, weak

pulse with narrow pulse pressure (<20mmHg (2.7 kPa), regardless of pressure levels, e.g. 100/90 mmHg (13.3/12.0 kPa)) or hypotension with cold, clammy skin and restlessness. As soon as the blood pressure or pulse, becoming imperceptible patients may pass into a stage of profound shock. The duration of shock is typically short as the patient dies within 12-24 hours, or recovers rapidly with prompt volume replacement therapy. Uncorrected shock can give rise to severe complications like metabolic acidosis, electrolyte imbalance, severe bleeding from the gastrointestinal tract and other organs. Intracranial haemorrhages can give rise to coma. Encephalopathy also has been reported occasionally. Physical examination or radiography may reveal pleural effusion and ascites. Survived patients of corrected DSS even in cases of profound shock may recover gradually within 2-3 days (WHO, 1997).

2.8: Pathogenesis of dengue

When dengue virus enters the body with the bite of an infected mosquito, it starts replication within the cells of mononuclear phagocytic lineage (macrophages, monocytes and B cells. Infection of mast cells, dendritic cells and endothelial cells is also known to occur (King et al., 2000; Ho et al., 2001). After the incubation period, viraemic phase starts when patient becomes febrile and infective (Malavige et al., 2004). Severity of dengue infection correlates with peak plasma viraemia that some patient may progress to leakage phase, leading to DHF and/or DSS (Libraty et al., 2002). There is differences in antibody, cytokine, and T-cell responses among the patients with uncomplicated dengue fever or DHD/DSS (Malavige et al., 2004).

• **Antibody responses to the dengue virus**:

Although only 2%-4% of individuals with a secondary dengue infections develop severe disease (MG, 2002), antibody dependent enhancement (ADE) mechanism has a great role to play in pathogenesis of severe dengue infections. When a patient becomes infected for the second time, pre-existing antibodies in his/ her body forms a complex with the second one in a way that results in more severe dengue, this is called ADE. In secondary dengue infections Fc portion of existed antibodies can bind to FcγRI and FcγRII bearing cells resulting in an increased number of cells being infected by dengue virus (Littaua et al., 1990). ADE depends on the presence of sub-neutralizing concentrations of dengue antibodies (Halstead,

1988). For example, DEN2 infection of mononuclear leucocyte (MNL) will be enhanced by the presence of DEN1 immune sera at 1:250 dilution (sub-neutralizing) titer, but not at 1: 10 dilution and this enhancement in turn results in increased lymphocyte production and decreased interferon-γ (IFN-γ) production (Yang et al., 2001). After primary dengue infection, antibodies that form against viral NS1 have been shown to induce endothelial cell apoptosis in a caspase dependent manner (Lin et al., 2002). Studies showed that, higher levels of dengue virus specific IgG1, IgG4 and lower levels of IgG2 are predominant in patients with DHF and DSS compared with those with dengue fever (Thein et al., 1993; Koraka et al., 2001). Besides, total and dengue specific IgE antibodies have been found to be higher in DHF and DSS (Koraka et al., 2003).

IgM type of antiplatelet antibodies, dengue viral specific antibodies, bone marrow hypocellularity (leading to defective megakaryocytes), or destruction of platelets in liver and spleen are considered to be responsible mechanisms causing various degrees of thrombocytopenia common in DHF (Hathirat et al., 1993). Increase in numbers of atypical lymphocytes is common in dengue infections. On the day of subsidence of fever, there is an increase in number of B-cells and a decrease in number of T-cells in DHF (Boonpucknavig et al., 1979). In addition, there is presence of anti-B cell antibodies in patients with DHF. All these are supposed to modulate humoral immune responses during infection (Gilbreath et al., 1983).

• Cytokine responses in dengue infections:

There are several studies explaining the mechanisms of cytokine responses in dengue viral infection. As soon as dengue virus replicates in macrophages, it quickly induces the CD4+ T cells to produce a unique cytokine, called human cytotoxic factor (hCF) (Chaturvedi et al., 2000). Under the influence of hCF, macrophages produce free radicals, nitrite, reactive oxygen and peroxynitrite (Misra et al., 1996; Misra et al., 1998). These free radicals not only kill the target cells by apoptosis but also directly up-regulate production of pro-inflammatory cytokines IL-1α, TNF-α, IL-8, and hydrogen peroxide in macrophages. As a result, there is change in the relative levels of protective IL-12 and TGF-α (transforming growth factor -α) which shifts a Th-1 dominant response to Th2-biased response resulting in an exacerbation of dengue disease and death of patients. Combined effect of histamine, free radicals, pro-

inflammatory cytokines and the products of the compliment pathway etc are responsible for increased vascular permeability. Thus, the hCF appears to be the key player in the pathogenesis of DHF; but, unfortunately what regulates its activity is unknown (Chaturvedi et al., 2000).

• Cellular responses in dengue infection:

When the monocytes and macrophages present dengue viral antigen on their surfaces to the memory T-cells, there is induction of proliferation and the production of pro-inflammatory cytokines such as IFN-γ and TNF-α.These cytokines can directly injure the vascular endothelial cells causing plasma leakage (Green et al., 1999; Rothman and Ennis, 1999). The magnitude of T-cell response correlates with disease severity (Loke et al., 2001; Zivna et al., 2002). studies show that, both sero-type specific and cross-reactive T-cell response can occur, with the later playing a role in severe disease arising from secondary infection (Stephenson, 2005). Study of dengue virus specific T-cell responses in Thai children by a researcher concluded that high levels of cell activation is associated with severe infection which is balanced by massive apoptosis and thus returns to normal levels on viral clearance (Mongkolsapaya et al., 2003). Further analysis of these cells showed that, there is relatively low affinity for the infecting virus serotype and higher affinity for another virus serotype, presumably from a previous infection (Stephenson, 2005). Actually, this observation suits the phenomenon of 'original antigenic sin' described many years ago (Fazekas de St and Webster, 1966), which admits that 'an antibody response to a secondary virus challenge is dominated by the activation of cross-reacting memory B-cells induced by the primary infection.' In secondary infection, these activated memory B-cells produce antibodies, which have low affinity towards the virus causing secondary infection. Therefore, there are high levels of T-cell activation, coupled with rapid death and the domination of cellular immune response by cells with a low affinity for the infecting virus. All these may suppress or delay virus clearance that ultimately leads to high viral loads and increased immunopathology (Stephenson, 2005).

2.9: Lab diagnosis of dengue viral infection

For early detection of severe cases, case confirmation and differential diagnoses with other infectious diseases, surveillance activities, outbreak control, pathogenesis, academic research, vaccine development and clinical trials; an efficient and accurate diagnosis of dengue is very much needed. A range of laboratory diagnostic methods for dengue virus infection to support patient management and disease control has been developed such as: detection of virus, viral nucleic acid, antigens or antibodies, or a combination of these techniques. During the early stages of the disease, presence of the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4-5 days. During this period virus isolation, nucleic acid or antigen detection methods are preferred, while after this period onward serology is the method of choice (WHO, 2009).

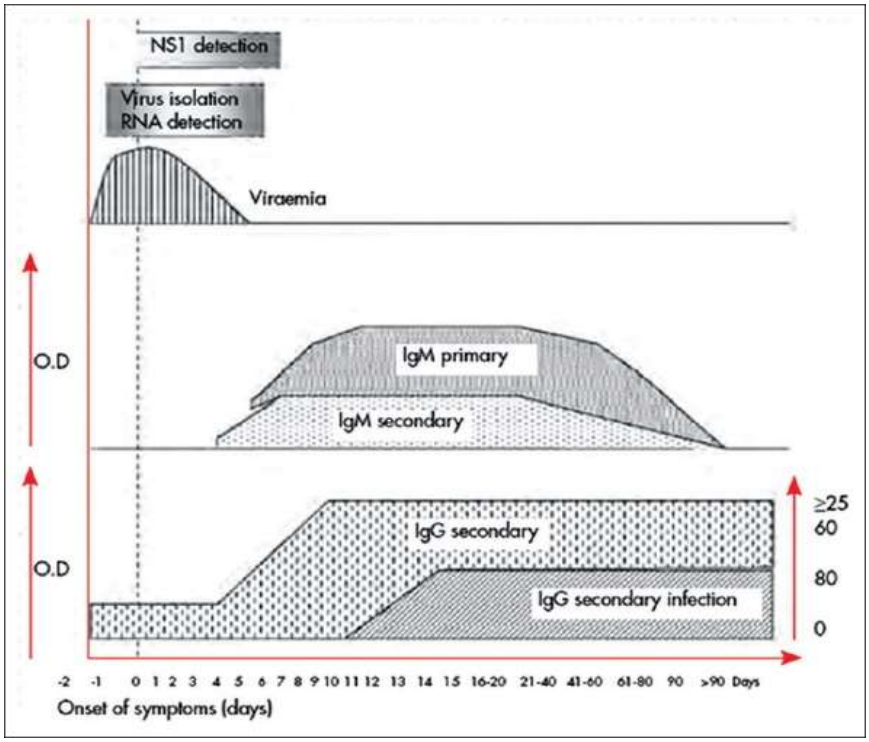

Figure 6.: Approximate time-line of primary and secondary dengue virus infections and the diagnostic methods that can be used to detect infection (WHO, 2009).

Current dengue diagnostic methods are:

♦**Virus isolation:** is more difficult and expensive but more specific than antibody detection by serology. During the period of viraemia (usually before day 5) dengue virus may be recovered from serum, plasma and peripheral blood mononuclear cells and also from tissues collected during autopsy (eg. liver, lungs, lymph nodes, thymus, bone marrow). There are 4 methods of viral isolation have been routinely used , such as: intracerebral inoculation of newborn mice, inoculation on mammalian cell cultures, intrathoracic inoculation of adult mosquitoes and inoculation on mosquito cell cultures (Guzmán and Kouri, 1996). Among these, mosquito cell culture is the most widely used method for dengue virus isolation(WHO, 2009) and C6/36 clone of A. albopictus cells are mostly used (Gubler et al., 1984). Screening must be done for specific evidence of infection by an antigen detection immunofluorescence assay using serotype specific monoclonal antibodies and flavi-virus group reactive or dengue complex-reactive monoclonal antibodies. All these require 1-2 weeks if associated other precautions during transport, storage, preservation of specimen secure the viability of virus in it (WHO, 2009).

♦ **Molecular Detection:** several new techniques have been developed for rapid diagnosis of dengue viruses by detection of its genome in acute phase of the disease and sometimes in convalescence phase.

✓ Nucleic acid hybridization: extracted RNA from either dengue virus-infected cell culture supernatants or pools of infected A. albopictus is hybridized with biotinylated probes or 32P- labelled probe. The detection method using radio labelled probes is more sensitive. It is used primarily in epidemiological studies and also can be used for viral diagnosis in tissues obtained in autopsies. It is not used as a routine method as working with RNA is a difficult task requiring highly sophisticated approach (De Paula and Fonseca, 2004).

✓Reverse transcription-Polymerase Chain Reaction (RT-PCR): Since 1990, RT-PCR assays have been developed in several way that detect and identify dengue serotypes. Result is more sensitive than virus isolation and varies from 80-100% (WHO, 2009). Nested RT-PCR assay; using universal dengue primers targeting the C/ prM region of the genome for an initial reverse transcription and amplification step followed by a nested PCR amplification that is serotype specific is now widely utilized in many

laboratories (Lanciotti et al., 1992). Another alternative to the nested RT-PCR is one-step multiplex RT-PCR is now also being used widely (Harris et al., 1998).

✓Real-time RT-PCR: is a one-step assay system that can quantitate viral RNA. Real-time RT-PCR assays either can detect only one serotype at a time (singleplex) or can identify all 4 serotypes form a single sample (multiplex). Multiplex real-time RT-PCR is faster but less sensitive than nested RT-PCR. Moreover, this method has the ability to determine viral titer in a clinical sample, which may be used to study the pathogenesis of dengue infection (WHO, 2009).

✓Isothermal amplification methods: the NASBA (nucleic acid sequence based amplification) is an example of isothermal RNA-specific amplification method. Its sensitivity is near about that of virus isolation in cell cultures and may be useful for studying dengue infection in field studies (WHO, 2009).

♦ Serological diagnosis: Hemagglutination inhibition (HI), complement fixation (CF), neutralization test (NT), immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA) and indirect immunoglobulin G ELISA are the five commonly used serological tests that have been used for the diagnosis of dengue infection (De Paula and Fonseca, 2004).

During both primary and secondary infection, specific antibodies IgM are produced temporarily and can be detected usually from the 5th day of disease and may persist for 60-90 days. Therefore, detection of Anti-dengue IgM antibodies in any serum samples within the last 2-3 months indicates active or recent infection. It is possible to distinguish primary infection from secondary by observing the level production of antibodies. In case of primary infection from the 5^{th} day of disease onset IgM is usually detected and from the 7^{th} days IgG is detected but in low levels. On the other hand, in secondary infection antibodies IgG are detected at high levels even in acute phase and antibodies IgM are generally detected in lower titers than those observed in the primary infections (WHO, 2009).

✓Hemagglutination inhibition test (HI): dengue antigens have the ability to agglutinate red blood cells (RBCs) of ganders or trypsinized humans O RBC and Anti-dengue antibodies in sera can inhibit this agglutination. HI measures the potency of this inhibition in paired sera obtained upon hospital admission (acute) and discharge (convalescent) or paired sera with an interval of more than 7 days. In

primary infection it shows slow increase in antibody titers after day 5 of disease onset and in secondary infection rapid increase usually exceeding 1:1280 in antibody titers (WHO, 2009). HIT provides high degree of sensitivity but low specificity and is not able to identify the infecting virus serotype (De Paula and Fonseca, 2004).

✓Complement fixation test (CF): complements are consumed during the antigen-antibody reaction. Complement fixing antibodies detected for CF appear after HI antibodies and persist for a shorter period. Though CF is fairly difficult to perform complement fixing antibodies are very specific in primary infection. It also helps in determining the infecting serotype in primary infection as monotypic responses are observed (De Paula and Fonseca, 2004).

✓Neutralization test (NT): Accurate determination of neutralization antibody titers is the most sensitive and specific serological tests for diagnosis of dengue infection, identifying serotype in primary infections and for vaccine trials (De Paula and Fonseca, 2004; Salje et al., 2014). Measurement of specific neutralizing antibodies by Plaque Reduction Neutralization Test (PRNT) is a key approach to differentiate dengue viral infection from other flavi viral infection. However, PRNT remains still costly and labor-intensive (Martinez et al., 2019).

✓Enzyme-linked immunosorbent assays (ELISAs): ELISA has become the most useful serological method for dengue diagnosis by detecting acute phase (IgM) and convalescent phase (IgG) antibodies as well as for the detection of antigens (Ag). IgM- antibody capture Enzyme-linked immunosorbent assay (MAC-ELISA) can rapidly detect the propagation of transmission during epidemics. IgG-ELISA technique is highly sensitive like HI but less specific. It can be useful in differentiation of primary and secondary infections by dengue but not in serotype identification (De Paula and Fonseca, 2004).

♦ Detection of antigens: until recently, in patients with secondary dengue infection, it was rare to detect antigens in acute phase sera as such patients had pre-existing virus-IgG antibody immune-complexes. Recent scientific enhancement demonstrated that, presence of high concentration of Envelope/ Membrane (E/M) antigen and the non-structural protein (NS1) in the form of immune-complexes can be detected in patients with both primary and secondary dengue infections up to 9 days after the onset of illness by ELISA and dot blot assays (WHO, 2009).

All flavivirus produce NS1 glycoprotein and it is also secreted from mammalian cells (WHO, 2009). The hexameric form of dengue NS1 antigen circulates in the sera during acute phase of illness (Kumarasamy et al., 2007a). It produces a very strong humoral response that early diagnosis of dengue viral infection is possible by using the detection of NS1 antigen (WHO, 2009). There is no cross-reaction of dengue NS-1 protein with those of other related flavi virus as it was not found in patients with Japanese Encephalitis Virus or Yellow Fever Virus infections (Lapphra et al., 2008). It is established that, plasma viraemia levels would be related to detection of plasma NS1 as it is a product of infected cells. NS1-negative patients have a significantly lower mean viraemia than NS1-positive patients. With the increasing time since the onset of symptoms, sensitivity of NS1 antigen detection declines as there is also a decline in viral burden (Kumarasamy et al., 2007b; Dussart et al., 2008). Its specificity is high during early infection, however a NS1 negative result does not rule out the diagnosis because, there may already have an established humoral immune response characteristic of a secondary infection and therefore, NS1 antigen test is more likely to be negative (Shrivastava et al., 2011). Several commercial kits are now available for the detection of NS1 and their performance is being utilized in peripheral laboratory worldwide and can yield result in a few hours (WHO, 2009).

2.10: Treatment

Symptomatic treatment is given, as there is no specific treatment for dengue fever. Antipyretics (fever reducers) and analgesics (pain killers) can be taken to control the symptoms such as: fever, muscle aches and pain, headache, eye pain, throat pain, joint pain. Acetaminophen or Paracetamol are the best option in this regard. Non-steroidal anti-inflammatory drugs (NSAIDs); for example, ibuprofen and aspirin should not be given as they may exacerbate the risk of hemorrhage (WHO, 2020). In case of severe dengue care, maintenance of patient's body fluid is the most important issue to save the patient (WHO, 2020). Normal saline, Ringer lactate, 5% glucose diluted 1:2 or 1:1 in normal saline, plasma, plasma substitutes, or 5% albumin are fluids of choice for volume expansion (Rajapakse et al., 2012). According to WHO guideline, crystalloid (0.9% saline) is the recommended first-line intravenous fluid and colloids (eg, dextran) are recommended as second-line fluids when hypotension is not responsive to boluses of intravenous crystalloids (WHO, 2009).

Strict monitoring should be taken during fluid management to keep enough fluid in vascular system during the leakage phase to avoid hypovolemia and while also to avoid fluid overloading. There is no exact defined platelet count at which platelet should be transfused. Usually, platelet transfusions are given to patients with serious haemorrhagic manifestations or have very low platelet counts (Rajapakse et al., 2012). According to WHO treatment guidelines, no clinical improvement despite the administration of sufficient fluids with a decrease in the haematocrit - e.g. from 0.5(50%) to 0.4 (40%) indicates significant internal haemorrhage. In this case, only enough volume of fresh whole blood is preferable to raise the red RBCs concentration to normal. Besides, massive bleeding in DIC Fresh frozen plasma and/or concentrated platelets also may be required (WHO, 2011). In addition to checking serial haematocrit and platelet levels some other tests are advised to evaluate patient conditions: serum electrolyte, blood gases, prothrombin time, partial thromboplastin time and thrombin time, liver function test (WHO, 2011).

2.11: Vaccination against dengue virus

Immunologic complexity, lack of an adequate animal model of disease, absence of an immune correlate of protection, and only partially informative immunogenicity assays are now top. most challenging issues in effective vaccine development against dengue virus (Thomas and Endy, 2013). A recombinant yellow fever-17D–dengue virus, live, attenuated, tetravalent dengue vaccine (CYD-TDV) has recently been tested for its efficacy and safety against symptomatic confirmed dengue during the active surveillance phase (up to 25 months after the first injection) of two phase III studies in 10 countries in Asia and Latin America. However, assessment of benefits expected from various vaccination strategies by various study models concluded that vaccination has the potential to significantly reduce the burden of dengue (Coudeville et al., 2016). This Dengvaxia® (CYD-TDV) is the first dengue vaccine developed by Sanofi Pasteur. It was licensed in December 2015 and recently has been approved by regulatory authorities in ~20 centuries. According to WHO, the results of an additional analysis were published in November, 2017, that retrospectively determined the sero-status at the time of vacation. The results demonstrated that there was higher risk of more severe dengue and hospitalizations from dengue in those trial participants who were supposed to be seronegative at the time of first vaccination compared to unvaccinated participants. For this instance, the

target people for using the vaccine are those residing in endemic areas, ranging from 9-45 years of age and most importantly, who have had at least 1 documented dengue viral infection previously (based on an antibody test, or on a documented laboratory confirmed dengue infection in the past). Effective vaccination is an integral part of dengue prevention and control strategy. Implementation of a pre-vaccination screening strategy is an earnest need in case of dengue elimination that will require careful assessment at the country level, including consideration of the sensitivity and specificity of available tests, and of local priorities, dengue epidemiology, country-specific dengue hospitalization rates, and affordability of both CYD-TDV and screening tests (WHO, 2020).

2.12: Prevention and control of Dengue viral infections

A dengue patient should avoid getting further mosquito bites during the first week of illness as virus may be circulating in the blood during this period and therefore he/she may transmit the virus to new uninfected mosquitoes, who may in turn infect other people (WHO, 2020). Safe, cost-effective, environmentally acceptable and appropriate methods (environmental, biological and chemical) are needed to adopt an integrated approach to mosquito control. Larval source reduction and successful, sustainable mosquito control program must involve a complete cooperation and partnership between government control agencies and the community to ensure community understanding and involvement in implementation (WHO, 2011).

♦Environmental Management: it focuses on minimizing vector breeding and thus reduction of humans-vector contact. According to WHO there are 3 kinds of environmental management:

1) Environmental modification: it involves vector habitats to be undergone long-lasting physical transformation. For example: improvement of water supply to reduce the necessity and use of water storage containers, using mosquito-proofing of overhead tanks/ cisterns or underground reservoirs.

2) Environmental manipulation: involves the management of “essential” and “nonessential” containers; and management or removal of “natural” breeding sites in order to temporarily change vector habitats. It can be brought by cleaning, covering,

storing under roof, modifying design, filling with sand or soil, collecting or recycling dispose, puncture or draining containers.

Table 6: Lists of essential and non-essential and natural water containers (WHO, 2011)

Essential	Non-essential	Natural
Water storage tank/cistern Drum (40-55 gal) Flower vase with water Potted plants with saucers Ornamental pool/fountain Roof gutter/sun shades Animal water container	Used tyres Discarded large appliances Discarded buckets Tin cans	Tree holes Rock holes

3) Changes to humans habitation or behavior: focuses on efforts to reduce man-vector-virus contact by changing habitation (such as designing building exterior that lacks the chance of providing holes and becoming mosquito habitat) or behavior (for example, periodic inspection of buildings during the rainy season to locate potential breeding sites). Personal protection such as wearing protective clothing, using household insecticidal products such as mosquito coils, mats, aerosols etc., mosquito repellents, sleeping under insecticide-treated mosquito nets and curtains (ITMN) are some excellent behavior towards mosquito control.

♦Biological Control: The application of biological control agents are mostly directed against the larval stages of dengue vectors.

Fish: in many countries of SEA, extensive use of larvivorus fish (Gambusiaaffinis and Poeciliareticulata) for the control of Ae. stephensi and/or Ae. aegypti in large water bodies or large water containers remains successful.

Bacteria: Bacillus thuringiensis serotype H-14 (Bt.H-14) and Bacillus sphaericus (Bs) are effective endotoxin-producing bacteria are being used as mosquito control agents. Bt. H-14 has an extremely low level of toxicity for mammals and has been found to be most effective against An. stephensi and Ae. aegypti, while Bs is the most effective against Culex quinquefasciatus (WHO, 2011).

Cyclopoids: copepod crustaceans have predatory role though lack of nutrients and frequent cleaning of some containers can prevent the sustainability of copepods. Therefore, they can be used in conjunction with Bt. H-14 in larger containers, which cannot be cleaned regularly.

♦Chemical control: oil, house fumigation with pyrethrins etc. were used to destroy larval habitats since the turn of the century. In 1940s, DDT was the major method of Aedes mosquito eradication but in early 1960s, resistance to DDT emerged. After that, organophosphate insecticides, including fenthion, malathion and fenitrothion were used for controlling adult mosquitoes. Currently, larvicide application and space spraying are two most widely used mosquito control strategies. Temephos 1% sand granules, Insect growth regulators (IGRs), Bacillus thuringiensis H-14 (Bt.H-14) are now three common and beneficial larvicide that can be used for treating containers that hold drinking water. Application of small droplets of insecticide into the air in an attempt to kill adult mosquitoes by space sprayers has been the principal method of DF/DHF control used by most countries in the Region for 25 years. Thermal fogging and Ultra-low volume (ULV) aerosols- cold fogs – mists are now two forms of space sprays and both can be spread by vehicle-mounted or hand operated machines (WHO, 2011).

Chapter 3: Materials and Methods

3.1: Description of the study area

Background: Savar, serving as a satellite town situated at a distance of about 29 km to the North-West of megacity Dhaka on the Dhaka-Aricha highway (Rahman et al., 2009). District Gazetteers published that, during 7th century AD Savar was the capital of Sambhog principality; the kingdom of Raza Harish Chandra. It then came into establishment in 1991 as a paurashava and now it is the most populous city of Dhaka Zila. Area and Location: the city stands on an area of 13.54 sq. km. and lies between 23º44′and 24º02′ North latitudes and between 90º11′and 90º22′ East longitudes.

Administrative/ Geographic Unit: The entire city is under the administrative control of Savar paurashava with 9 wards and 56 mahallas.

Population Characteristics: The total humans' population of the city is 296851 with a density of 21924 persons per sq. km. There are 157018 males and 139833 females living in total 75902 households and the sex ratio is 112. Literacy rate (7 years and above) is 74.9% (BBS, 2014).

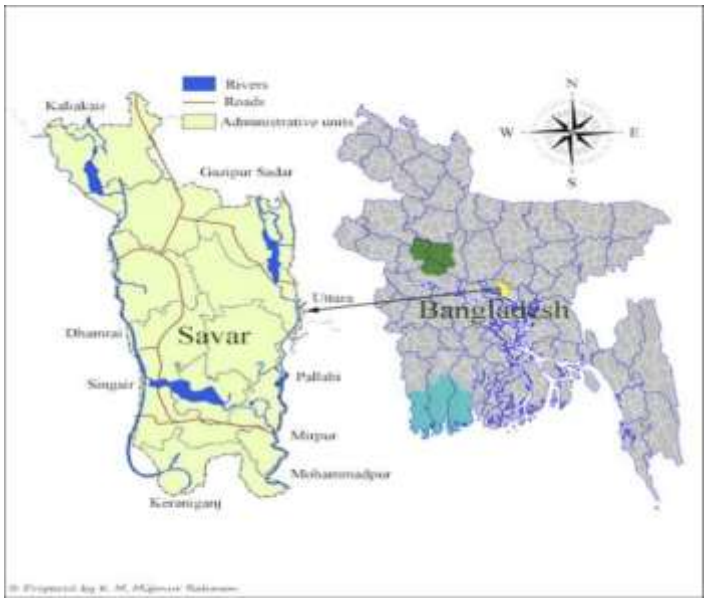

Figure 7: Map of the study area.

Climate: Savar is an example of prevailing tropical savanna climate. It has average annual temperature 32 ºC and with the highest average of 37 ºC in April and the lowest is 26 ºC in January. It has about 507 mm of rainfall in a year with an average humidity of 67% (climate).

3.2 Study design :

A cross sectional study was done in 2 medical hospitals of Savar, Dhaka to investigate the sero-epidemiology of dengue virus in this part of the country. Hospital record book was used to collect data of the patients for this study. Sample was selected according to the case definition.

3.3 Case definition:

WHO's case definitions for dengue fever

According to the 1997 WHO guideline, dengue is classified into dengue fever (DF), dengue hemorrhagic fever (DHF) (Grades 1 and 2) and dengue shock syndrome (DSS) (DHF Grades 3 and 4). The international DENCO study evaluated several limitations of the 1997 definition that led to a new WHO classification in 2009, which classified dengue according to levels of severity:

- dengue without warning signs
    - probable dengue
        - live in/travel to dengue endemic area
        - fever and 2 of the following criteria:
            - nausea, vomiting
            - rash
            - aches and pain
            - tourniquet test positive
        - any warning sign
    - laboratory confirmed dengue (important when no sign of plasma leakage)

- dengue with warning signs - requires strict observation and medical intervention
    - dengue as defined above with any of the following:
        - abdominal pain or tenderness
        - persistent vomiting
        - clinical fluid accumulation (ascites, pleural effusion)
        - mucosal bleeding
        - lethargy, restlessness
        - liver enlargement >2 cm
        - laboratory – increase in Haematocrit concurrent with rapid decrease in platelet count
- severe dengue - dengue with at least one of the following criteria:
    - severe plasma leakage leading into:
        - shock (DSS)
        - fluid accumulation with respiratory distress
    - severe bleeding – as evaluated by clinician
    - severe organ involvement
        - liver: AST or ALT ≥ 1000
        - CNS: impaired consciousness
        - failure of heart and other organs (WHO, 2009; Hadinegoro, 2012).

3.4: Study period

All hospital data of the suspected patients and laboratory results were collected over the period of 12 months (January to December) in 2019.

3.5: Sample Collection

A total 449 suspected patients from 2 hospitals of Savar were selected for this study. After formalities of registration and ensuring primary treatment blood samples were collected for laboratory investigations. For lab diagnosis of dengue, blood samples were collected by venipuncture in Evacutainer tubes without containing anticoagulants during first 3 days of onset of clinical symptoms or from 6 days onwards from the onset of clinical symptoms. Blood samples were then left for 30 minutes to coagulate and then centrifuged 3000 rpm for 10 minutes to separate the serum supernatants. Sterile 1.5 ml Eppendrof tubes were used to store the individual serum sample for testing. Individual labeling was used and stored the entire serum sample in the laboratory on 4ºC for further processing.

3.6: Data collection

A detailed data was collected in predetermined questionnaire from the patients at the time of history taking, from clinical findings and relevant lab results. The questions were aimed to see the association between the variables with dengue virus in Savar area of Dhaka. The variables were comprised with Season, Location, Sex, Age, Weight, Body temperature on admission, Platelet count, Serological findings, Days of hospital stay and Outcome. Seasons were divided into 3 types (summer: March to June; rainy: July to October and winter: November to February) (Bangladesh Bureau of Statistics (BBS), 1994. Statistical Pocket Book of Bangladesh, (Statistic Division, Government of the People's Republic of Bangladesh). Location is subdivided into Urban and Peri-urban areas. There were 4 types of age categories of the patients (≤15 years, 16-30 years, 31-50 years and ≥51 years).

3.7: Serological analysis

In this study, we used to detect the evidence of dengue viral infection through detection of dengue NS1 antigen, anti-dengue IgM antibody and anti-dengue IgG antibody by using commercially available immune chromatographic test (ICT) kits. Dengue NS1 antigen was detected by using HUMASIS Dengue NS1 Antigen immune chromatographic diagnostic kit (manufacturer: Humasis Co,. Ltd. Gyeonggi-do,

14042, Republic of Korea; authorized representative: MT Promedt Consulting GmbH, (St. Ingbert/ Germany)). Antibodies were detected by SD BIO-LINE Dengue IgM/IgG WB ICT diagnostic kit (Standard Diagnostics, INC. Gyeonggi-do, Republic of Korea; authorized representative: MT Promedt Consulting GmbH, (St. Ingbert/ Germany)). All positive samples were evaluated and categorized clinically for proper management. Positive samples were stored in (-) 4ºC for further analysis.

3.7.1: One step Dengue NS1 Antigen test

Procedure: After pulling out the specimen and device, it was left on room temperature for 15 minutes before the test. After 15 minutes, sealed pouch was opened and the test device was taken out. 3 drops (100 micro liter) of serum was taken by a dropper and was dropped in specimen insertion-hole. In 15-20 minutes the result was read cautiously. Reading after 20 minutes may yield false results.

Interpretation of results:

1. Positive: 2 colored bands visible in the test region (T) and control region (C).

2. Negative: A colored band is visible only in the control region (C).

3. Invalid: No colored band in control region (C). Or, no colored band in either control region (C) or test region (T). It may be due to deterioration of the test device or improper test procedure. It requires repeating the test with a new kit.

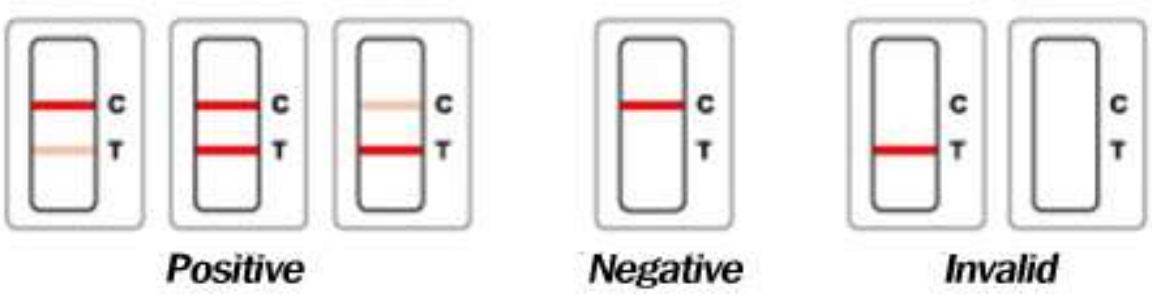

Figure 8: Interpretation of NS1 test result.

3.7.2: One step rapid test for detection of IgM/IgG antibodies to dengue virus

Procedure: all kit components and samples were brought to reach room temperature (between 15ºC and 30º C) prior testing. Test device was removed from the foil pouch and placed on a flat dry surface. The test device was labeled with a patient identifier. Immediately after opening the device, 10 microliter of serum was dispensed into the square specimen well marked "S" by using a micropipette. 4 drops (about 90-120 microliter) of assay diluent was dispensed into the round assay diluent well. Interpretation was done in 15-20 minutes. Reading after 20 minutes may yield false results.

Interpretation:

1) Negative: presence of only control line ("C") in the result window indicates that IgM and IgG antibodies to dengue virus are not present in the sample or are present below the detectable levels.

2) IgM positive: the presence of 2 colored lines ("C" and "M") in the result window, regardless of which line appears first, indicates that the sample is positive for IgM antibodies to dengue virus. This suggests a primary dengue infection. Test is positive even if "M" line is faint.

3) IgG positive: the presence of 2 colored lines ("C" and "G") in the result window, regardless of which line appears first, indicates that the sample is positive for IgG antibodies to dengue virus. This suggests a secondary or past dengue infection. Test is positive even if "G" line is faint.

4) IgG and IgM positive: the presence of 3 colored lines ("C", "M" and "G") in the result window, regardless of which line appears first, indicates that the sample is positive for both IgM and IgG antibodies to dengue virus. This suggests a late primary or early secondary dengue infection. Test is positive even if "M" and/or "G" line(s) are faint.

5) Invalid: no control line ("C") in the result window means the test is invalid, whether or not the test line ("M" and/or "G") is present. It requires to repeat the test with a new kit.

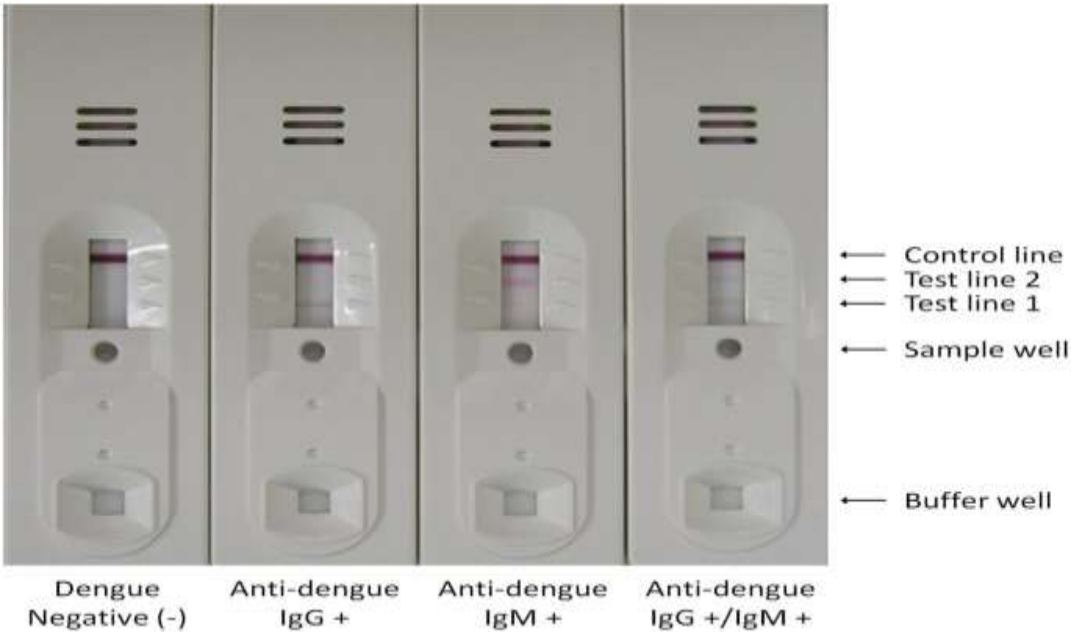

Figure 9: interpretation of antibody test result

3.8: Statistical analysis

Data from questionnaire and laboratory testing results were entered, collated, coded and stored in Microsoft Excel spreadsheet version 2016. For statistical analysis, statistical software: STATA/IC 13 (StataCorp 4905, Lakeway Drive, College Station, Texas 77845, USA) was used.

3.8.1 Descriptive analysis

Prevalence of dengue virus was calculated using positive samples divided by the total number of samples tested and the results were expressed as a percentage with 95% confidence interval (CI). Month wise prevalence of the dengue virus was calculated to observe the outbreak. Prevalence of dengue virus according to the sex within the month was presented by bar diagram.

3.8.2 Risk factor analysis

Univariable analyses were conducted for each of the independent variables (potential risk factors collected in the questionnaires) against the dependent variable (infection status of the patient). Chi square test was done to identify significant risk factors for the presence of dengue virus. The variables: Season, Location, Sex, Age,

Weight, Temperature on admission, Platelet count, Serological results were found significant ($p<0.05$) by chi-square test for the prevalence of dengue virus.

3.8.3 Logistic regression model

All variables ($p<0.2$) was forwarded to logistic regression model after chi sqaure test variable: location was omitted due to collinearity. After adjusting the factor, season, sex, age, weight, temperature on admission, platelet count, serological results were found as significant risk factor. Confounder was checked by considering the coefficient variation more than 10%. The validity of the model checked by receiver operating characteristic curve (ROC) and goodness of fit test (Dohoo et al., 2003). The result of multiple logistic regression model expressed by odds ratio (OR), 95% confidence interval and P value.

Chapter 4: Result

To accomplish the goal of the study, we collected data from two hospital facilities of Savar area under Dhaka district over a year time period. Suspected Dengue infected patients enrolled into hospitals and samples were selected according to the case definition. A total 449 patients were selected over one year time frame and serum samples were tested against Dengue virus antigen and 329 samples showed positive as Dengue virus. Overall 60% patients were identified as positive for NS1 antigen from EMC with 95% CI (53.9-67.2). The percentage was 73.8% in case of UHC patients with 95% CI (67.5-79.4). From antibody perspective, 5.8% patients showed positive against IgM antibody with 95% CI (3.1-9.7) from EMC, whereas, this percentage was slightly higher (6.7%) from UHC cohort with 95% CI. The results were shown almost similar pattern for IgG antibodies. We found 5.8% positive results in both hospital facilities. The urban and semi-urban group both were being affected with dengue virus in much consistence percentage in our study findings. The percentage was 67.7% in both occasions with 95% CI (61.6-73.5: 59.6-73.2). IgM positive dengue prevalence from urban area was 6.4% with 95% CI (3.7-10.1), from peri-urban area was 6.1% with 95% CI (3.2-10.3). The trends were same for IgG positive dengue prevalence from urban area which was 5.6% with 95% CI (3.1-9.2), from peri-urban area was 6.1% with 95% CI (3.2-10.3).

4.1: Prevalence of dengue virus by month

In each month patient came to the hospital with the signs and symptoms of dengue viral infection except February. In the outbreak season (April to October), the prevalence was higher than the other months which produce an epidemic curve. Among all the months, the proportionate prevalence of dengue virus (NS1 Ag positive) was highest in April 87.5%; (95% CI: 73.2-95.8) followed by September, proportionate prevalence is 84%; (95% CI: 63.9-95.5) and October 80%; (95% CI: 28.4-99.5), and in August, July and June was 77.2%, 71.4% and 70.4% respectively. In case of IgM positive patients, highest proportionate prevalence was found in October 20%; (95% CI: 0.5-71.6) followed by July 17.1%; (95% CI: 6.6-33.6). Moreover, in IgG positive dengue patients, highest proportionate prevalence was found in October 20%; (95% CI: 0.5-71.6) followed by December 13.3%; (95% CI:

1.7-40.5). No positive dengue cases were found during January, February and March (figure 11)**.**

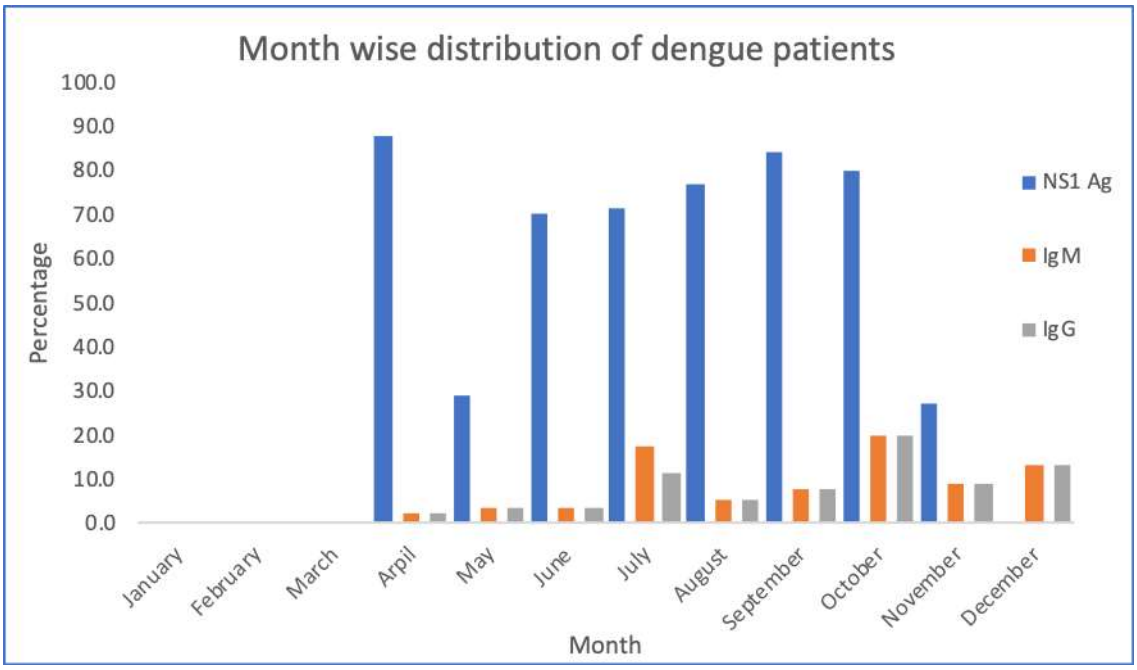

Figure 10: Month wise distribution of NS1 positive dengue patients in percentage.

4.2 Gender wise distribution and platelet count of dengue positive patients

The male and female were affected 69.9% and 30.1%, respectively from the samples collected in two hospital facilities. Among them 0.73% male and 0.64% female cases had <20,000 per cmm of platelet count in their laboratory blood picture. 11.68% male and 11.54% female patients had platelet count on an average 21,000-50,000 per cmm of blood. Percentage of male dengue positive patients having platelet count between 51,000-100,000 per cmm of blood was higher (45.99%) than the female (32.37%) while females having platelet count >10,000 per cmm of blood was higher (55.45%) than the male dengue positive patients (41.61%).

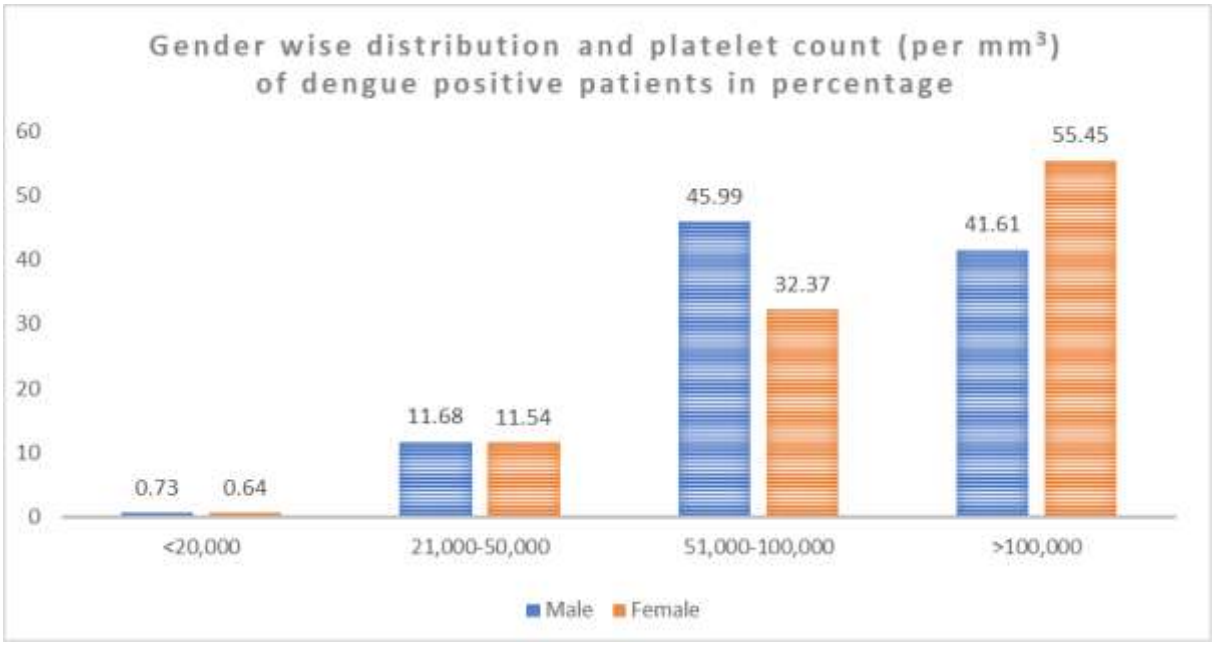

Figure 11: Gender wise distribution and platelet count of dengue positive patients

4.3: Age group wise distribution and platelet count of dengue positive patients

We categorized 4 age groups in this study samples. Patients of ≤15 years age group were predominantly seen to have platelet count of 51,000-100,000 per cmm of blood in their blood picture (51.35%) and equal percentage of this age group (9%) had platelet count ranging 21,000-50,000 and >100,000 per cmm of blood. Among the patients of age group 16-30; 52.52% had their platelet count >100,000 per cmm of blood, 38.66% of patients had platelet count 51,000-100,000 per cmm of blood and only 8.82% of the patients of this age group had platelet count ranging from 21,000 to 50,000 per cmm of blood in their blood picture. Besides, age groups 31-50 years and ≥51years had platelet count >100,000 per cmm of blood 56.55% and 48.28% of the patients respectively. Only 1.38% patients of 31-50 years of age group had their platelet count <20,000 per cmm of blood. 11.72% and 30.34% patients of age group 31-50 years had their platelet count 21,000-50,000 and 51,000-100,000 per cmm of blood respectively. Age group ≥51 years had 3.45% patients having platelet count <20,000 per cmm of blood and 17.24% and 31.03% of patients respectively had platelet count 21,000-50,000 and 51,000-100,000 per cmm of blood. As a whole, highest percentage of the study population (51.22%) had their platelet count >100,000

per cmm of blood, second highest percentage (36.53%) had platelet count 51,000-100,000 per cmm of blood. 11.58% of population had platelet count ranging 21,000-50,000 and only 0.67% had <20,000 per cmm of blood.

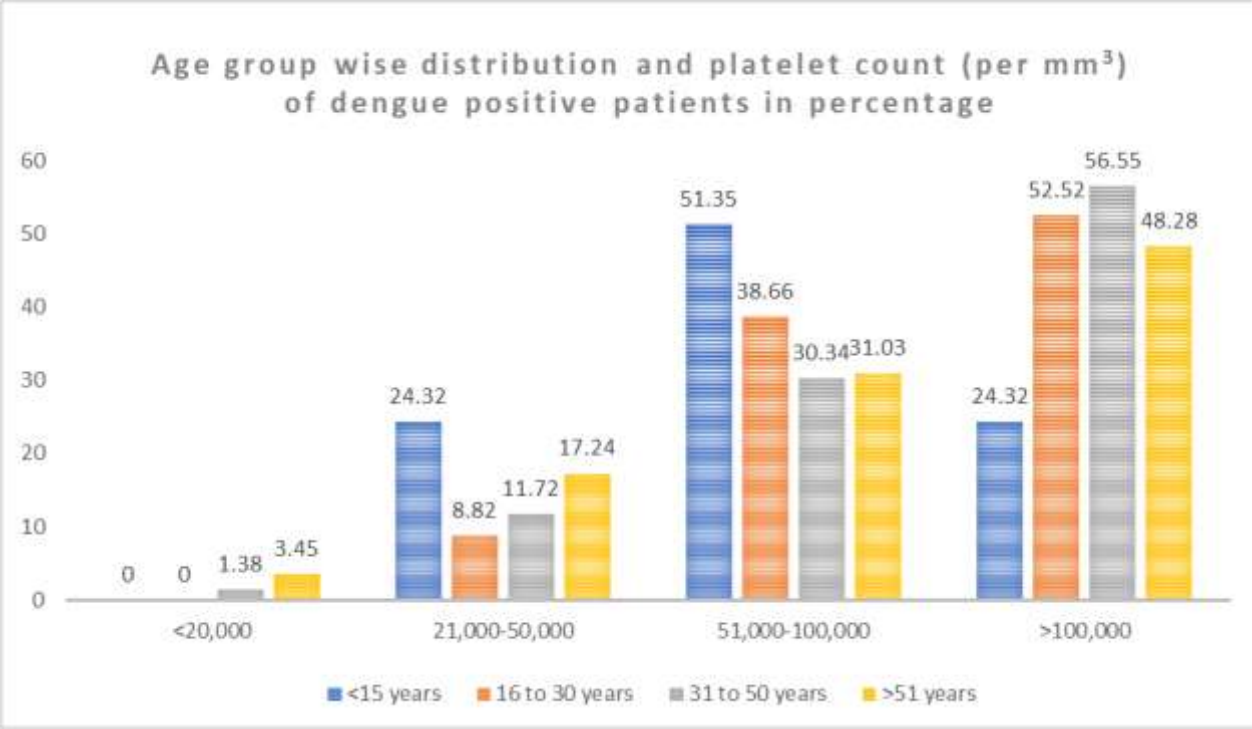

Figure 12: Age group wise distribution and platelet count

4.4: Body temperature wise distribution and platelet count (per cmm) of dengue positive patients in percentage:

Highest percentage of the population (52.08%) had platelet count >10,000 per cmm when suffered from 100-102ºF temperature during admission followed by 50% of patients had the temperature over 102ºF and around 48.05% patient had temperature below 100ºF. Among the patients who had platelet count between 51,000-10,00,00 cmm of blood, about 41.67% had been recorded to have >102ºF body temperature, 36.31% had 100-102ºF and 35.06% had <100ºF. The group of the patients who had 21,000-50,000 per cmm of blood showed that, 15.58% had <100ºF temperature, 11.01% had 100-102ºF temperature and 8.33% patients had >102ºF

temperature. In addition, among the patients who had <20,000 per cmm of blood had 1.3% and 0.6% were recorded for having <100ºF and 100-102ºF, respectively.

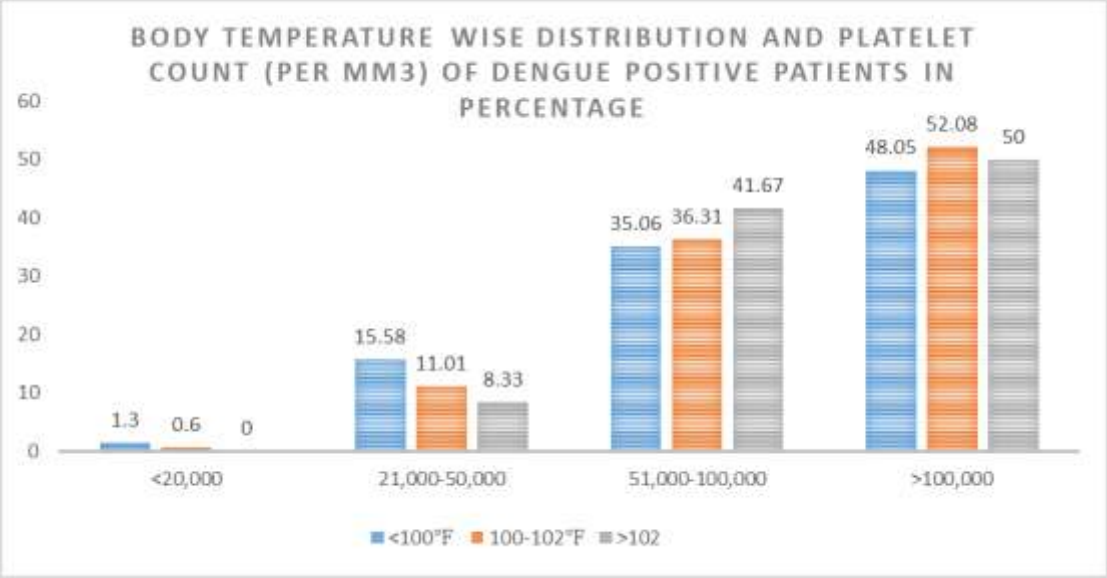

Figure 13: Body temperature wise distribution and platelet count (per cmm) of dengue positive patients in percentage

4.5: Univariate analysis of dengue viral infection for NS1 Ag test in Savar

To know the association of different categories of the variables with the presence of dengue virus, univariate analysis for NS1 Ag, anti-dengue IgM antibody and anti-dengue IgG antibody were performed by chi square tests. P value <0.05 was considered as significant. The prevalence of dengue was found significantly higher (p<0.00) in rainy season (76.5%; 95% CI: 71.5-81.1) than the summer (52.04%; 95% CI: 41.7-62.2) and winter season (11.1%; 95% CI: 2.4-29.2) (Table 1). Considering areas, the seroprevalence of dengue virus was significantly higher (P=0.812) in urban area (67.7%; 95% CI: 61.6-73.5) where as in peri-urban area it is lower (66.7%; 95% CI: 59.6-73.2). Age groups 16-30 years and ≥51 years were highly infected that measured by chi square test (68.9%; 95% CI: 62.6-74.7) and (68.9%; 95% CI: 49.2-84.7) compare to other age group. Seroprevalence of dengue in ≤15 years of age group was 62.2%; 95% CI: 44.8-77.5 and in 31 to 50 years of age group was 65.5%; 95% CI: 57.2-73.2.

Table 7: Univariate analysis of dengue viral infection for NS1 Ag test

Factor	Categories	N	n (%)	95% CI	P
Institution	Enam Medical College	224	136 (60.7)	53.9-67.2	0.003
	Upazilla Health Complex	225	166 (73.8)	67.5-79.4	
Season	Summer	98	51 (52.04)	41.7-62.2	<0.001
	Rainy	324	248 (76.5)	71.5-81.1	
	Winter	27	3 (11.1)	2.4-29.2	
Landscape	Urban	251	170 (67.7)	61.6-73.5	0.812
	Peri-urban	198	132 (66.7)	59.6-73.2	
Age	≤15 years	37	23 (62.2)	44.8-77.5	0.809
	16 to 30 years	238	164 (68.9)	62.6-74.7	
	31 to 50 years	145	95 (65.5)	57.2-73.2	
	≥51 years	29	20 (68.9)	49.2-84.7	
Sex	Male	312	210(67.3)	61.8-72.5	0.974
	Female	137	92 (67.2)	58.6-74.9	

The findings of the study revealed a higher association (p=0.974) between the male patient and dengue viral infection. Seroprevalence of dengue in male patient was 67.3%; 95% CI: 61.8-72.5 and in female 67.2%; 95% CI: 58.6-74.9.

4.6: Univariate analysis of dengue viral infection for anti-dengue IgM antibody test in Savar.

Chi- square test showed, it was winter when there was higher infection (p=0.228); prevalence was 11.1%; 95% CI: 2.3-29.2. In rainy season, it was 6.8%; 95% CI: 4.3-10.1 and in summer it was 3.1%; 95% CI: 0.6-8.7. In urban area prevalence of infection (p= 0.891) was higher (6.4%; 95% CI: 3.7-10.1) than the peri-urban area (6.1%; 3.2-10.3%).

Table-8: Univariate analysis of dengue viral infection for anti-dengue IgM antibody test

Factor	**Categories**	**N**	**n (%)**	**95% CI**	**P**
Institution	Enam Medical College	224	13 (5.8)	3.1-9.7	0.705
	Upazilla Health Complex	225	15 (6.7)	3.8-10.8	
Season	Summer	98	3 (3.1)	0.6-8.7	0.228
	Rainy	324	22 (6.8)	4.3-10.1	
	Winter	27	3 (11.1)	2.3-29.2	
Landscape	Urban	251	16 (6.4)	3.7-10.1	0.891
	Peri-urban	198	12 (6.1)	3.2-10.3	
Age	≤15 years	37	1 (2.7)	0.06-14.2	0.680
	16 to 30 years	238	17 (7.1)	4.2-11.2	
	31 to 50 years	145	9 (6.2)	2.9-11.5	
	≥51 years	29	1 (3.5)	0.08-17.8	
Sex	Male	312	20 (6.4)	3.9-9.7	0.818
	Female	137	8 (5.8)	2.6-11.2	

Age group 16-30 years had been higher infection which was around 7.1%; (95% CI: 4.2-11.2) (p=0.680).The next susceptible age group were 31-50 years with 6.2% infection (95% CI: 2.9-11.5). The two opposite end age groups ≤15 years and ≥51 years had prevalence of IgM positive dengue infection 2.7%; 95% CI: 0.06-14.2 and 3.5%; 95% CI: 0.08-17.8, respectively. Male population was prone to be infected much higher percentage (6.4%; 95% CI: 3.9-9.7) than the female counterpart (5.8%; 95% CI: 2.6-11.2)

4.7: Univariate analysis of dengue viral infection for anti-dengue IgG antibody test in Savar

In winter season there was higher prevalence of IgG positive dengue viral infection (11.1%; 95% CI: 2.4-29.2). In rainy and summer seasons the prevalence was 6.2%; 95% CI: 3.8-9.3 and 3.1%; 95% CI: 0.6-8.6 respectively. The prevalence of IgG positive infection (p=0.228) was higher in peri-urban areas (6.1%; 95% CI: 3.2-10.3) than the urban settings (5.6%; 95% CI: 3.1-9.2)

Table-9: Univariate analysis of dengue viral infection for anti-dengue IgG antibody test

Factor	Categories	N	n (%)	95% CI	P
Institution	Enam Medical College	224	13 (5.8)	3.1-9.7	0.991
	Upazilla Health Complex	225	13 (5.8)	3.1-9.7	
Season	Summer	98	3 (3.1)	0.6-8.6	0.228
	Rainy	324	20 (6.2)	3.8-9.3	
	Winter	27	3 (11.1)	2.4-29.2	
Landscape	Urban	251	14 (5.6)	3.1-9.2	0.891
	Peri-urban	198	12 (6.1)	3.2-10.3	
Age	≤15 years	37	1 (2.7)	0.06-14.2	0.576
	16 to 30 years	238	17 (7.1)	4.2-11.1	

	31 to 50 years	145	7 (4.8)	1.9-9.7	
	≥51 years	29	1 (3.5)	0.08-1.8	
Sex	Male	312	19 (6.1)	3.7-9.3	0.818
	Female	137	7 (5.1)	2.1-10.2	

Among the four age groups, 16-30 years of age group were produced higher IgG antibodies which was around 7.1% that demonstrate the previous infection (95% CI: 4.2-11.1) than other age groups. Prevalence of the infection in 31-50 years of age group was 4.8%; 95% CI: 1.9-9.7. ≥51 years and ≤15 years of age groups had the prevalence 3.5%; 95% CI: 0.08-1.8 and 2.7%; 95% CI: 0.06-14.2 respectively. We found considerably more male being were admitted into the hospitals (prevalence 6.1%; 95% CI: 3.7-9.3) than the female counterpart (5.1%; 95% CI: 2.1-10.2).

Chapter 5: Discussion

In Bangladesh perspective; climate change, unplanned rapid urbanization and construction, massive population densities, and the insufficient health-care system further accelerate the magnitude and severity of dengue outbreaks (Hasan, 2019). Unusual elevated dengue prevalence with recurrent outbreaks in Bangladesh is the result of dengue virus transmission through the routes of SEA countries, which is the results of increasing movement of population and goods through by air, roads etc. In recent times, a massive influx of refugees from Myanmar (where large scale outbreaks of dengue is recorded in each year) places Bangladesh at risk of imported dengue cases though such incidents have not been recorded yet (Tabassum and Taylor-Robinson, 2019). The current governments are tried to isolate these vulnerable population in a separate island named Basan Chor. The aims of this migration are to keep this population in control and save the biodiversity in Cox's bazar.

Dengue viral infections may be asymptomatic or may give rise to undifferentiated fever, dengue fever, DHF, or dengue shock syndrome with serious complications like Encephalopathy and encephalitis, Liver failure, Myocarditis, Disseminated intravascular coagulation leading to massive bleeding (Malavige et al., 2004).Dengue is predominantly found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. Interestingly, in this study, we investigated the dengue virus epidemiology and assessing the condition in urban and semi-urban perspective. It is a great concern that the Dhaka, which has become a megacity of 20 million inhabitants. However, this massive population increases pressure on construction facilities that promote new high-rise buildings, the avenue of potential areas for dengue outbreak inside the construction sites.

In 1964, the first ever dengue cases were reported in Bangladesh and the outbreak was popularly known as 'Dacca Fever' (Pervin et al., 2017). From 1964-1999 sporadic transmissions recorded to occur but in 2000, the first officially recorded significant outbreak had occurred by DENV-3 (Mamun et al., 2019; Tabassum and Taylor-Robinson, 2019). Since then, several outbreaks have been observed in different years with different serotypes such as in 2016 with DENV-2, in

2015-2017 with DENV-1 and DENV-2 and absence of DENV-3 and 4 (Tabassum and Taylor-Robinson, 2019). DENV-1 and DENV-2 strains have been circulating in the country for more than a decade and presumably, large percentage of the population is supposed to be immuned to these circulating serotypes. Thus, outbreaks of severe dengue cases in 2018 and 2019 correlate with the prevalence of serotype DENV-3 (Akram, 2019).

However, some previous studies show the sero-prevalence and outbreak records of dengue in several locations of Bangladesh but we do not have epidemiological insights and genomics features in certain areas specially that are close to Megacity, Dhaka (Pervin et al., 2017)**.** Thus, this is the first study of this type that investigates this scenario at Savar, Dhaka and it might enhance the ongoing control plan against future outbreak of dengue in this part of the world. Additionally, the current study might provide some substantial information into the sero-prevalence of dengue virus and associated risk factors in this area of Bangladesh, where population densities and new construction is underway. A nationwide seroprevalence study (2014–2015) showed that, in Bangladesh around 24% of the participants had past history of dengue infection and this sero-positivity was highly confined to three major big cities, namely Dhaka, Chittagong, and Khulna. It would further raise the possibility that dengue virus circulation was not as high in the semi-urban and rural areas (like Savar) as we expected. Similarly, other major dengue outbreaks, the 2019 outbreak initially confined mostly in Dhaka city. Its peak point been observed in the month of August and thereafter the outbreak diffuses in rural areas as well (Hossain et al., 2020a).

This study revealed that the proportionate prevalence of dengue was higher in April (87.5%) and subsequently this became a decreasing trend up to August; though in the middle part of the year the proportionate percentage were the lowest margin. In September, proportionate prevalence was 84%, in October 80%, in August 77.2%. The prevalence of dengue in July is 71.4%, in June 70.4%. The numbers of cases were zero in the month of January, February, March and December in our study. Similar study from India, identified the October month when the peak of dengue infection was highest in subsequent two years' time period (2014 and 2015) (Biradar et al., 2016). In contrast, a study in Thailand showed that from 2003 to 2017, peak of dengue endemic occurred in November 2015, which was very much unusual and

might cause significant error for prediction. Thus, it may indicate the possibility of dengue cases being occurred at varying times each year (Polwiang, 2020).

In 2019 outbreak, 57% (n=7952) of all cases were reported prior to EId Ul Adha compared to the rest of the part of the country (43%, n=5927). However, during Eid and post Eid vacation number of cases of outside Dhaka started to rise and within the Dhaka, it started to fall. At the initial stage, the outbreak was mostly localized in Dhaka, the city area and then gradually affected almost all districts of the country. Approximately 51,179 cases were located within Dhaka city and 49,022 cases had been identified from rest of the country, during January to December implying a 10 fold increase in hospitalized case than the largest outbreak before 2019. Around 4-7 fold net increase was observed in some districts of Bangladesh, including Narail, Pirojpur, Manikgonj and Faridpur (Hossain et al., 2020a).

In this study, a total of 224 patients from a medical college hospital (EMC) and 225 patients from a Upazilla health complex (Savar UHC) were enrolled over one year time frame. The serum samples were tested against dengue antigen and antibodies to identify the seroprevalence. Overall, 302 patients (136 from EMC and 166 patients from UHC) were positive for Dengue NS1 Ag test. The samples collected from medical hospital showed lower prevalence compare to the health complex. The prevalence of NS1 positive Dengue from EMC was 60.7% with 95% CI (Range: 53.9-67.2), from UHC was 73.8% with 95% CI (Range: 67.5-79.4). A total of 28 patients (13 from EMC and 15 from UHC) were developed IgM and IgG antibodies and only 2 patients showed recent infection. Immunoglobulins, IgM were found in 5.8% patients with 95% CI (3.1-9.7) from EMC and this figure was 6.7% with 95% CI (3.8-10.8) from UHC. In contrast, IgG positive dengue prevalence from EMC was 5.8% with 95% CI (3.1-9.7) from UHC 5.8% with 95% CI (3.1-9.7). When patients got admitted within 1-3 days of onset of fever, dengue NS1 antigen was detected in patients' serum for confirming the diagnosis. In contrast, antibodies were detected only when patients came after 4-5 days of onset of fever. This might contributed to the lower percentage of antibody positive cases as the positive cases being enrolled in the hospital in early stage of infection. The literacy percentage is very high (74.9%) (Above 7 years) among population in this areas (BBS, 2014). However, different government and non-government implement awareness campaign could play an important role in early admission of people suffering with high-grade

fever and other warning signs. In addition, NGO's authority provides the treatment expenses for their staff might play a significant role to have developed health consciousness among this group of population.

Of the total 449 patients 120 (26.73%) were negative for dengue NS1 antigen or antibody tests. This suggests the possibility of other organisms of similar clinical manifestations may also prevalent in our study area which requires in depth investigations though a negative NS1 antigen test or antibody test does not exclusively rule out the positivity due to less specificity of the tests (De Paula and Fonseca, 2004; Shrivastava et al., 2011). In 2017, there was a massive outbreak of Chikungunya supporting the assumption of fever with similar clinical manifestations. It occurred at the similar season as dengue and was being spread by the common mosquito vector; *Aedes aegypti* (Khatun et al., 2015). Unfortunately, detection of Chikungunya or other related viruses could not be possible. Those were either treated as cases of 'dengue like illness' or other causes of high grade fever with similar sign-symptoms, laboratory results were evaluated thoroughly and treated accordingly. A study in Dhaka Medical College carried on 2017 showed that, 45% of the 145 suspected patients admitted into that hospital was positive for NS1 and 47.5% was positive for IgM antibodies (Pervin et al., 2017). Also, another study in 2000 showed a very high (92%) positivity of antibody test among the patients in Dhaka city (Pervin et al., 2004).

This study showed that young adults (16-30 years) were more infected than other age groups. However, both age group 16-30 years and ≥51 years were equally positive for NS1 antigen (68.9%), IgM and IgG positive cases were higher among the young adults. The nationwide study on 2019 dengue outbreak also showed that younger adults aged 15–35 years accounted for 51·42% of 29 855 total cases (Mamun et al., 2019). Previous study from 2000 also found that majority of the cases were of 20-29 years of age (Pervin et al., 2004). Studies from other countries surrounding Bangladesh observed the similar patterns of prevalence percentage (Ooi, 2001). Different studies unambiguously proposed that in primary and secondary infections; the conditional risks of clinical attack increases as a function of the age. Thus the adolescents and young adults are more likely to suffer from symptomatic dengue than younger ones (Thai et al., 2011). Besides, 'the successful vector control and surveillance program resulted in lowered seroprevalence and herd immunity in the

adult population, while the shift in acquisition patterns that was outside of the home (due to better vector elimination in residential versus non-residential sites), resulted in the acquisition of disease at a later stage in life (Lin et al., 2017). On the other hand, though the severity of dengue is more in elderly patients due to age related impairment of physiological and immunological system, increased probability of acquiring secondary dengue and increased prevalence of chronic diseases and other co-morbidities; patients aged over 60 were significantly less likely to report fever, or have leucopenia at the time of presentation (Lye et al., 2010; Lin et al., 2017). Therefore,, according to the current guidelines a significant proportion of dengue in the elderly would likely to be missed (Lin et al., 2017).

In 2019 outbreak exhibited an imminent predominance in men (64·11% for men *vs* 35·89% for women), and primarily encompassed younger adults (whereby people aged 15–35 years accounted for 51·42% of 29 855 total cases) (Mamun et al., 2019). Our study also showed male was infected with dengue in highest percentage which was about 69.9% and 30.1% was their female counterpart. Some other studies in other parts of the country and the rest of the world also found the similar trends of male predominance in occurrence of the infection (Ooi, 2001; Sharmin et al., 2015; Pervin et al., 2017). However, in case of dengue outbreak 2000, there was no such evidence of difference in sex among dengue cases in Dhaka (Pervin et al., 2004). Also, studies in South America showed that, there was either equal proportions of male and female dengue cases or a greater proportion of female cases (Pervin et al., 2017). Therefore, it is not clear to predict that, this difference may indicate more susceptibility of male to dengue infection. In this subcontinent females are less privileged so that they can't seek medical treatment only for fever (Pervin et al., 2017). Another reason may be that males are more exposed to dengue-carrying mosquitoes during daytime hours either at workplace or while travelling to and from work (Ooi, 2001). Also, working adults who are more likely to be male in Asian countries may be more likely to seek treatment and be reported to authority when ill because they need medical certification for absences (Anker and Arima, 2011).

In UHC, 80.9% male patient was positive, of which 97.9% was positive for NS1 antigen test, 7.1% and 6.3% patients were positive by anti-dengue IgM and anti-dengue IgG antibody, respectively. Among 68 female patients in UHC, proportionate prevalence of positive patients was 79.4% of which 88.9% was positive for antigen

test and 11.1% and 9.3% was positive against IgM and IgG antibodies. In contrast, among 66.5% positive male patients, 89.3% was positive for antigen test, 10.7% was positive for anti-dengue IgM test and 10.7% was found positive for anti-dengue IgG test in EMC. For the opposite gender of patients in EMC, 65.2% were found positive of which 97.8% was positive for dengue NS1 antigen test, 4.4% patients were positive for antibody tests.

Continuous drizzling, high temperature and humidity are the suitable environmental condition that favors mosquitoes breeding. In tropical zones, these conditions prevail in pre monsoon (March- May) and post monsoon (June-October) periods. In previous outbreaks, August was the month found as the peak season of Dengue (Pervin et al., 2004; Morales et al., 2016). However, since 2014 more dengue cases have been reported during pre-monsoon season. During 2015-2017, dengue cases were found seven times higher in pre-monsoon season compared to the previous 14 years (Mutsuddy et al., 2019). In our study, we found cases all over the pre monsoon and post monsoon seasons. In April, we found the highest proportionate prevalence (87.5%) that matches other recent studies. Also, according to metrological data, in Savar highest recorded temperature prevails in April (37º C) (climate) which suits this higher percentage of cases there in Savar. Next in September it was 84% and in October 80%. In August, proportionate prevalence was 77.2%. Such high prevailing rate in both pre-monsoon and post-monsoon seasons was possibly due to the climate change that might short the rainy season and intermittent rain might occur during post-monsoon season. An absolute platelet count on a given day during the febrile phase is considered to be an important risk factor for developing DSS. In addition, a change in the platelet count of a patient over time also implies a risk to the likelihood of developing DSS. Another important clinical aspect is a higher baseline temperature, which has been revealed to be independently associated with an elevated risk of DSS. Furthermore, it has been established as a finding which might be explained by the positive correlation between fever and viral load (Lam et al., 2017). In our study, patients who had platelet count 21000-50,000 per cmm of blood, only 8.33% of them had body temperature >102ºF. It may be due to the fact that, both body temperature and viral load decrease with the advances of time (Tsai et al., 2013) though the condition of the patients is deteriorating due to plasma leakage.

Chapter 6: Conclusion

An emerging disease, Dengue; like present time it will continue to constitute a serious public health problem in Bangladesh as is happening worldwide. This study represents the presence of Dengue virus in Savar Upazila of Dhaka district in Bangladesh and appears to be one of the few studies to evaluate the sero-prevalence of Dengue virus from Savar, Bangladesh. This study findings agreed with other similar studies carried out in different geographical region both national and international areas. Percentage of antigen (NS1) found higher compared to antibodies (IgM/ IgG). April, September and October were the peak season of the infection. Adult age group (16-30 years) were more infected than other. Male was higher infected than female. Substantial percentage of suspected patients in this study were negative by dengue tests suggesting other infections of similar clinical presentations that are becoming prevalent in Bangladesh. To understand the rapidly changing epidemiology of dengue to control and prevent further epidemic constant monitoring is needed, including extending the surveillance areas and addressing the challenges to reduce the impact of the disease on public health and on the economy of the country. It may be very challenging to tear up by the root of this endemic disease from the country. Multi sectorial approach under the umbrella of One Health is a current need to involve everyone to join the fight against the *Aedes* mosquitoes.

Chapter 7: Limitations

The study was conducted only with two selective hospitals and hospitalized patients, so there was absolute chance to miss dengue infected patients those are diagnosed other hospital or clinics or some patients might not come to the hospital. Due to taking only data from hospitals, the results did not represent the actual prevalence of Dengue Virus. Though all the data were collected from hospital record books there are missing of important variables (such as specific environmental, virological and humans behavioral practice) those might be associated with the infection and outbreak. In case of testing procedure, only rapid antigen and antibody detection tests were used but in modern age polymerase chain reaction (PCR) is more authentic. We also could not exclude Chikungunya, other mosquito borne Flavivirus and Alphavirus infections due to our limited resource. Budget and time were the main constraints.

Chapter 8: Recommendations

Dengue virus remains a substantial threat to increased morbidity and mortality worldwide, predominantly in the developing countries. Reasons are multi-factorial. Despite of significant disease, social and economic burden that it possesses; dengue has been described as a neglected tropical disease because of historical lack of coordinated efforts, political will and research attention. To reduce the burden of dengue it has been prioritized via WHO's Global Strategy for Dengue Prevention and Control, 2012–2020. Without the presence of definitive therapeutic strategies and effective vaccine against Dengue virus Integrated Vector Control (IVM) should be the most effective and essential choice to control mosquitoes. Surveillance, reporting and more accurate quantification of the impact of dengue globally will allow improved political, financial, and research prioritization as well as informed decision making and enhanced modeling to combat Dengue virus effectively.

References:

Akram, A., 2019. Alarming turn of dengue fever in Dhaka city in 2019. Bangladesh Journal of Infectious Diseases 6, 1-2.

Amin, M., Hussain, A., Nahar, K., Chowdhury, I., Murshed, M., Chowdhury, S., 2000. Searo-diagnosis of Dengue Infections in Four Metropolitan Cities of Bangladesh.

Anderson, J.R., Rico-Hesse, R., 2006. *Aedes aegypti* vectorial capacity is determined by the infecting genotype of dengue virus. American Journal of Tropical Medicine and Hygiene 75, 886-892.

Anker, M., Arima, Y., 2011. Male–female differences in the number of reported incident dengue fever cases in six Asian countries. Western Pacific Surveillance and Response Journal: WPSAR 2, 17.

Ashburn, P.M., Caraig, C.F., 1907. Experimental investigations regarding the etiology of dengue fever. The Journal of Infectious Diseases, 440-475.

Åström, C., Rocklöv, J., Hales, S., Béguin, A., Louis, V., Sauerborn, R., 2012. Potential distribution of dengue fever under scenarios of climate change and economic development. Ecohealth 9, 448-454.

Barrett, A.D., Stanberry, L.R., 2009. Vaccines for biodefense and emerging and neglected diseases. Academic Press.

Barthel, A., Gourinat, A.-C., Cazorla, C., Joubert, C., Dupont-Rouzeyrol, M., Descloux, E., 2013. Breast milk as a possible route of vertical transmission of dengue virus? Clinical Infectious Diseases 57, 415-417.

Baum, A., Sachidanandam, R., García-Sastre, A., 2010. Preference of RIG-I for short viral RNA molecules in infected cells revealed by next-generation sequencing. Proceedings of the National Academy of Sciences 107, 16303-16308.

BBC, 2019. ডেঙ্গু জ্বর: ঈদে বাড়ি যাবে লক্ষ মানুষ, স্বাস্থ্য ঝুঁকির আশঙ্কা কতটা? , BBC News Bangla.

BBS, 2014. Bangladesh Population and Housing Census 2011, National Report: Volume—3, Urban Area Report. Bangladesh Bureau of Statistics, Statistics and Informatics Divisions ….

Biradar, A., Kauser, Y., Itagi, I., Jamadar, N.A., 2016. Dengue infection: its prevalence with seasonal variations. Indian Journal of Microbiology Research 3, 89-92.

Bisimwa, P., 2015. Mosquito abundance and molecular detection of arvoviruses in Kyela district, Tanzania.

Boonpucknavig, S., Vuttiviroj, O., Bunnag, C., Bhamarapravati, N., Nimmanitya, S., 1979. Demonstration of dengue antibody complexes on the surface of platelets from patients with dengue hemorrhagic fever. American Journal of Tropical Medicine and Hygiene 28, 881-884.

Boulton, R., Westaway, E., 1976. Replication of the flavivirus Kunjin: proteins, glycoproteins, and maturation associated with cell membranes. Virology 69, 416-430.

Brinton, M., 1986. Replication of flaviviruses. In "The Togaviridae and Flaviviridae"(S. Schlisenger, and M. Schlisenger, Eds.). Plenum Press, New York.

Burke, D.S., Nisalak, A., Johnson, D.E., Scott, R.M., 1988. A prospective study of dengue infections in Bangkok. American Journal of Tropical Medicine and Hygiene 38, 172-180.

Carrington, L.B., Armijos, M.V., Lambrechts, L., Scott, T.W., 2013. Fluctuations at a low mean temperature accelerate dengue virus transmission by *Aedes aegypti*. PLOS Neglected Tropical Diseases 7, e2190.

CDC, 2019a. Clinical presentation of Dengue viral infection.

CDC, 2019b. Treatment of Dengue viral infection.

Chambers, T.J., McCourt, D.W., Rice, C.M., 1989. Yellow fever virus proteins NS2A, NS213, and NS4B: Identification and partial N-terminal amino acid sequence analysis. Virology 169, 100-109.

Chaturvedi, U., Agarwal, R., Elbishbishi, E., Mustafa, A., 2000. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunology & Medical Microbiology 28, 183-188.

Chen, H.-R., Lai, Y.-C., Yeh, T.-M., 2018. Dengue virus non-structural protein 1: a pathogenic factor, therapeutic target, and vaccine candidate. Journal of Biomedical Science 25, 58.

Chen, L.H., Wilson, M.E., 2004. Transmission of dengue virus without a mosquito vector: nosocomial mucocutaneous transmission and other routes of transmission. Clinical Infectious Disease 39, e56-60.

Chu, P., Westaway, E., 1985. Replication strategy of Kunjin virus: evidence for recycling role of replicative form RNA as template in semiconservative and asymmetric replication. Virology 140, 68-79.

Cleaves, G.R., Ryan, T.E., Schlesinger, R.W., 1981. Identification and characterization of type 2 dengue virus replicative intermediate and replicative form RNAs. Virology 111, 73-83.

climate, W.a., Best time to visit, weather and climate Savar.

Coudeville, L., Baurin, N., L'Azou, M., Guy, B., 2016. Potential impact of dengue vaccination: Insights from two large-scale phase III trials with a tetravalent dengue vaccine. Vaccine 34, 6426-6435.

Cummings, D.A., Iamsirithaworn, S., Lessler, J.T., McDermott, A., Prasanthong, R., Nisalak, A., Jarman, R.G., Burke, D.S., Gibbons, R.V., 2009. The impact of the demographic transition on dengue in Thailand: insights from a statistical analysis and mathematical modeling. PLOS Medecine 6, e1000139.

Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.-Y., Schneller, S., Zust, R., Dong, H., 2010. 2′-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature 468, 452-456.

Daughaday, C., Brandt, W., McCown, J., Russell, P., 1981. Evidence for two mechanisms of dengue virus infection of adherent human monocytes: trypsin-sensitive virus receptors and trypsin-resistant immune complex receptors. Infection and Immunity 32, 469-473.

Davidson, S.S., 2018. Dengue virus.

De Paula, S.O., Fonseca, B.A.L.d., 2004. Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis. Brazilian Journal of Infectious Diseases 8, 390-398.

De Souza, S.S., da Silva, I.G., da Silva, H.H.G., 2010. Association between dengue incidence, rainfall and larval density of *Aedes aegypti*, in the State of Goiás. Revista da Sociedade Brasileira de Medicina Tropical 43.

DGHS, 2020. No new dengue case in last 24 hrs. The financial Express.

Dick, O.B., San Martín, J.L., Montoya, R.H., del Diego, J., Zambrano, B., Dayan, G.H., 2012. The history of dengue outbreaks in the Americas. The American Journal of Tropical Medicine and Hygiene 87, 584-593.

Duong, V., Lambrechts, L., Paul, R.E., Ly, S., Lay, R.S., Long, K.C., Huy, R., Tarantola, A., Scott, T.W., Sakuntabhai, A., Buchy, P., 2015. Asymptomatic humans transmit dengue virus to mosquitoes. Proceedings of the National Academy of Sciences of the United States of America 112, 14688-14693.

Dussart, P., Petit, L., Labeau, B., Bremand, L., Leduc, A., Moua, D., Matheus, S., Baril, L., 2008. Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum. PLOS Neglected Tropical Diseases 2, e280.

El Sahili, A., Lescar, J., 2017. Dengue Virus Non-Structural Protein 5. Viruses 9.

Endy, T.P., Chunsuttiwat, S., Nisalak, A., Libraty, D.H., Green, S., Rothman, A.L., Vaughn, D.W., Ennis, F.A., 2002a. Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. American Journal of Epidemiology 156, 40-51.

Endy, T.P., Nisalak, A., Chunsuttiwat, S., Libraty, D.H., Green, S., Rothman, A.L., Vaughn, D.W., Ennis, F.A., 2002b. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Kamphaeng Phet, Thailand. American Journal of Epidemiology 156, 52-59.

Fahad, S., Khan, L., Iqbal, A., Khan, I., Umar, A., Muneer, S.H., 2018. Institutional based prevalence and occurrence of dengue disease in capital city Peshawar of province Khyber Pakhtunkhwa (KPK), Pakistan.

Fazekas de St, G., Webster, R.G., 1966. Disquisitions of Original Antigenic Sin. I. Evidence in man. Journal of Experimental Medicine 124, 331-345.

Ferreira, G.L., 2012. Global dengue epidemiology trends. Revista do Instituto de Medicina Tropical de São Paulo 54, 5-6.

Filomatori, C.V., Lodeiro, M.F., Alvarez, D.E., Samsa, M.M., Pietrasanta, L., Gamarnik, A.V., 2006. A 5′ RNA element promotes dengue virus RNA synthesis on a circular genome. Genes & Development 20, 2238-2249.

Gilbreath, M., Pavanand, K., MacDermott, R., Ussery, M., Burke, D., Nimmannitya, S., Tulyayon, S., 1983. Cold-reactive immunoglobulin M antilymphocyte

antibodies directed against B cells in Thai children with dengue hemorrhagic fever. Journal of Clinical Microbiology 17, 672-676.

Gollins, S.W., Porterfield, J.S., 1986. A new mechanism for the neutralization of enveloped viruses by antiviral antibody. Nature 321, 244-246.

Green, S., Vaughn, D.W., Kalayanarooj, S., Nimmannitya, S., Suntayakorn, S., Nisalak, A., Lew, R., Innis, B.L., Kurane, I., Rothman, A.L., 1999. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. The Journal of Infectious Diseases 179, 755-762.

Grun, J.B., Brinton, M.A., 1987. Dissociation of NS5 from cell fractions containing West Nile virus-specific polymerase activity. Journal of Virology 61, 3641-3644.

Gubler, D., Kuno, G., Sather, G., Velez, M., Oliver, A., 1984. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. The American Journal of Tropical Medicine and Hygiene 33, 158-165.

Gubler, D.J., 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. Dengue and dengue hemorrhagic fever.

Gubler, D.J., 2011. Dengue, urbanization and globalization: the unholy trinity of the 21st century. Tropical Medicine and Health 39, S3-S11.

Gubler, D.J., Clark, G.G., 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. Emerging Infectious Disease 1, 55-57.

Gubler, D.J., Suharyono, W., Tan, R., Abidin, M., Sie, A., 1981. Viraemia in patients with naturally acquired dengue infection. Bull World Health Organ 59, 623-630.

Gulshan, P., 2019. Story of the Aedes mosquito. Dhaka Tribune.

Gupta, R.K., Gupta, G., Chorasiya, V.K., Bag, P., Shandil, R., Bhatia, V., Wadhawan, M., Vij, V., Kumar, A., 2016. Dengue Virus Transmission from Living Donor to Recipient in Liver Transplantation: A Case Report. Journal of Clinical and Experimental Hepatology 6, 59-61.

Guzmán, M.G., Kouri, G., 1996. Advances in dengue diagnosis. Clinical and diagnostic laboratory immunology 3, 621.

Hadinegoro, S.R.S., 2012. The revised WHO dengue case classification: does the system need to be modified? Paediatrics and International Child Health 32, 33-38.

Hahn, Y.S., Caller, R., Hunkapiller, T., Dalrymple, J.M., Strauss, J.H., Strauss, E.G., 1988. Nucleotide sequence of dengue 2 RNA and comparison of the encoded proteins with those of other flaviviruses. Virology 162, 167-180.

Hales, S., De Wet, N., Maindonald, J., Woodward, A., 2002. Potential effect of population and climate changes on global distribution of dengue fever: an empirical model. The Lancet 360, 830-834.

Halstead, S., 2008. Tropical Medicine: Science and Practice. Dengue, volume 5. Imperial College Press.

Halstead, S.B., 1980. Immunological parameters of togavirus disease syndromes. The togaviruses. biology, structure, replication. academic press New York, 174.

Halstead, S.B., 1988. Pathogenesis of dengue: challenges to molecular biology. Science 239, 476-481.

Halstead, S.B., Scanlon, J.E., Umpaivit, P., Udomsakdi, S., 1969. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. The American Journal of Tropical Medicine and Hygiene 18, 997-1021.

Hammon, W.M., Rundnick, A., Sather, G., 1960. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. Science 131, 1102-1103.

Hanley, K.A., Weaver, S.C., 2010. Frontiers in dengue virus research. Caister Academic Press.

Harris, E., Roberts, T.G., Smith, L., Selle, J., Kramer, L.D., Valle, S., Sandoval, E., Balmaseda, A., 1998. Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. Journal of Clinical Microbiology 36, 2634-2639.

Hasan, M.K., 2019. Dengue outbreak shatters all records. Dhaka Tribune.

Hase, T., Summers, P., Eckels, K., 1989. Flavivirus entry into cultured mosquito cells and human peripheral blood monocytes. Archives of Virology 104, 129-143.

Hase, T., Summers, P., Eckels, K., Baze, W., 1987. Maturation process of Japanese encephalitis virus in cultured mosquito cells in vitro and mouse brain cells in vivo. Archives of Virology 96, 135-151.

Hathirat, P., Isarangkura, P., Srichaikul, T., Suvatte, V., Mitrakul, C., 1993. Abnormal hemostasis in dengue hemorrhagic fever. The Southeast Asian Journal of Tropical Medicine and Public Health 24, 80.

Henchal, E.A., Putnak, J.R., 1990. The dengue viruses. Clinical Microbiology Reviews 3, 376-396.

Ho, L.-J., Wang, J.-J., Shaio, M.-F., Kao, C.-L., Chang, D.-M., Han, S.-W., Lai, J.-H., 2001. Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. The Journal of Immunology 166, 1499-1506.

Holmes, E.C., 2009. RNA virus genomics: a world of possibilities. The Journal of Clinical Investigation 119, 2488-2495.

Hossain, M.S., Siddiqee, M.H., Siddiqi, U.R., Raheem, E., Akter, R., Hu, W., 2020a. Dengue in a crowded megacity: Lessons learnt from 2019 outbreak in Dhaka, Bangladesh. PLOS Neglected Tropical Diseases 14, e0008349.

Hossain, M.S., Siddiqee, M.H., Siddiqi, U.R., Raheem, E., Akter, R., Hu, W., 2020b. Dengue in a crowded megacity: Lessons learnt from 2019 outbreak in Dhaka, Bangladesh. PLOS Neglected Tropical Diseases. e0008349.

Idrees, S., Ashfaq, U.A., 2012. A brief review on dengue molecular virology, diagnosis, treatment and prevalence in Pakistan. Genetic Vaccines and Therapy 10, 6.

Iglesias, N.G., Gamarnik, A.V., 2011. Dynamic RNA structures in the dengue virus genome. RNA Biology 8, 249-257.

Irie, K., Mohan, P., Sasaguri, Y., Putnak, R., Padmanabhan, R., 1989. Sequence analysis of cloned dengue virus type 2 genome (New Guinea-C strain). Gene 75, 197-211.

Kalayanarooj, S., 2011. Clinical manifestations and management of dengue/DHF/DSS. Tropical medicine and health, 1112080193-1112080193.

Kalayanarooj, S., Vaughn, D.W., Nimmannitya, S., Green, S., Suntayakorn, S., Kunentrasai, N., Viramitrachai, W., Ratanachu-Eke, S., Kiatpolpoj, S., Innis, B., 1997. Early clinical and laboratory indicators of acute dengue illness. Journal of Infectious Diseases 176, 313-321.

Khatun, S., Chakraborty, A., Rahman, M., Nasreen Banu, N., Rahman, M.M., Hasan, S.M., Luby, S.P., Gurley, E.S., 2015. An outbreak of chikungunya in rural Bangladesh, 2011. PLOS Neglected Tropical Diseases 9, e0003907.

Kimura, T., Gollins, S.W., Porterfield, J.S., 1986. The effect of pH on the early interaction of West Nile virus with P388D1 cells. Journal of General Virology 67 (Pt 11), 2423-2433.

King, C.A., Marshall, J.S., Alshurafa, H., Anderson, R., 2000. Release of vasoactive cytokines by antibody-enhanced dengue virus infection of a human mast cell/basophil line. Journal of Virology 74, 7146-7150.

Koraka, P., Murgue, B., Deparis, X., Setiati, T.E., Suharti, C., van Gorp, E.C., Hack, C., Osterhaus, A.D., Groen, J., 2003. Elevated levels of total and dengue virus-specific immunoglobulin E in patients with varying disease severity. Journal of Medical Virology 70, 91-98.

Koraka, P., Suharti, C., Setiati, T., Mairuhu, A., Van Gorp, E., Hack, C., Juffrie, M., Sutaryo, J., Van Der Meer, G., Groen, J., 2001. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. Journal of Clinical Microbiology 39, 4332-4338.

Kularatne, S.A., 2015. Dengue fever. British Medical Journal 351, h4661.

Kumarasamy, V., Chua, S., Hassan, Z., Wahab, A., Mohamad, M., Chua, K., 2007a. Evaluating the sensitivity of a commerical dengue NS1 antigen-capture ELISA for early diagnosis of acute dengue virus infection. Singapore Medical Journal 48, 669.

Kumarasamy, V., Wahab, A.A., Chua, S., Hassan, Z., Mohamad, M., Chua, K., 2007b. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. Journal of Virological Methods 140, 75-79.

Kweka, E.J., Baraka, V., Mathias, L., Mwang'onde, B., Baraka, G., Lyaruu, L., Mahande, A.M., 2018. Ecology of Aedes mosquitoes, the major vectors of Arboviruses in human population. Dengue Fever-A resilient threat in the face of innovation, 39-56.

Kyle, J.L., Harris, E., 2008. Global spread and persistence of dengue. Annual Review of Microbiology 62, 71-92.

Lam, P.K., Ngoc, T.V., Thu Thuy, T.T., Hong Van, N.T., Nhu Thuy, T.T., Hoai Tam, D.T., Dung, N.M., Hanh Tien, N.T., Thanh Kieu, N.T., Simmons, C., 2017. The value of daily platelet counts for predicting dengue shock syndrome: Results from a prospective observational study of 2301 Vietnamese children with dengue. PLOS Neglected Tropical Diseases 11, e0005498.

Lambrechts, L., Paaijmans, K.P., Fansiri, T., Carrington, L.B., Kramer, L.D., Thomas, M.B., Scott, T.W., 2011. Impact of daily temperature fluctuations on dengue

virus transmission by *Aedes aegypti*. Proceedings of the National Academy of Sciences of the United States of America 108, 7460-7465.

Lanciotti, R.S., Calisher, C.H., Gubler, D.J., Chang, G.-J., Vorndam, A.V., 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. Journal of Clinical Microbiology 30, 545-551.

Lapphra, K., Sangcharaswichai, A., Chokephaibulkit, K., Tiengrim, S., Piriyakarnsakul, W., Chakorn, T., Yoksan, S., Wattanamongkolsil, L., Thamlikitkul, V., 2008. Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. Diagnostic Microbiology and Infectious Disease 60, 387-391.

Leary, K., Blair, C.D., 1980. Sequential events in the morphogenesis of Japanese encephalitis virus. Journal of Ultrastructure Research 72, 123-129.

Levinson, W., 2016. Review of Medical Microbiology and Immunology.

Libraty, D.H., Young, P.R., Pickering, D., Endy, T.P., Kalayanarooj, S., Green, S., Vaughn, D.W., Nisalak, A., Ennis, F.A., Rothman, A.L., 2002. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. The Journal of Infectious Diseases 186, 1165-1168.

Lin, C.-F., Lei, H.-Y., Shiau, A.-L., Liu, H.-S., Yeh, T.-M., Chen, S.-H., Liu, C.-C., Chiu, S.-C., Lin, Y.-S., 2002. Endothelial cell apoptosis induced by antibodies against dengue virus nonstructural protein 1 via production of nitric oxide. The Journal of Immunology 169, 657-664.

Lin, R.J., Lee, T.H., Leo, Y.S., 2017. Dengue in the elderly: a review. Expert Review of Anti-infective Therapy 15, 729-735.

Littaua, R., Kurane, I., Ennis, F.A., 1990. Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. The Journal of Immunology 144, 3183-3186.

Loke, H., Bethell, D.B., Phuong, C., Dung, M., Schneider, J., White, N.J., Day, N.P., Farrar, J., Hill, A.V., 2001. Strong HLA class I–restricted T cell responses in dengue hemorrhagic fever: a double-edged sword? The Journal of Infectious Diseases 184, 1369-1373.

Lumsden, W., 1957. The activity cycle of domestic Aedes (Stegomyia) aegypti (L.)(Dipt., Culicid.) in southern province, Tanganyika. Bulletin of Entomological Research 48, 769-782.

Lye, D., Lee, V., Sun, Y., Leo, Y., 2010. The benign nature of acute dengue infection in hospitalized older adults in Singapore. International Journal of Infectious Diseases 14, e410-e413.

Mahalingam, S., Herring, B.L., Halstead, S.B., 2013. Call to action for dengue vaccine failure. Emerging Infectious Diseases 19, 1335.

Mahmud, F., 2019. Why is Bangladesh grappling with a record dengue fever outbreak? , Al Jazeera.

Malavige, G., Fernando, S., Fernando, D., Seneviratne, S., 2004. Dengue viral infections. Postgraduate Medical Journal 80, 588-601.

Mamun, M.A., Misti, J.M., Griffiths, M.D., Gozal, D., 2019. The dengue epidemic in Bangladesh: risk factors and actionable items. The Lancet 394, 2149-2150.

Mandl, C.W., Guirakhoo, F., Holzmann, H., Heinz, F.X., Kunz, C., 1989. Antigenic structure of the flavivirus envelope protein E at the molecular level, using tick-borne encephalitis virus as a model. Journal of Virology 63, 564-571.

Markoff, L., 1989. In vitro processing of dengue virus structural proteins: cleavage of the pre-membrane protein. Journal of Virology 63, 3345-3352.

Martinez-Torres, E., Polanco-Anaya, A., Pleites-Sandoval, E., 2008. Why and how children with dengue die. Revista cubana de.

Martinez, J.D., Cardenas-de la Garza, J.A., Cuellar-Barboza, A., 2019. Going viral 2019: Zika, chikungunya, and dengue. Dermatologic Clinics 37, 95-105.

Mason, P.W., McAda, P.C., Mason, T.L., Fournier, M.J., 1987. Sequence of the dengue-1 virus genome in the region encoding the three structural proteins and the major nonstructural protein NS1. Virology 161, 262-267.

Maswood, M.H., 2020. Dengue shakes health system. New Age Bangladesh.

MG, G., 2002. Kouri G. Dengue: an update. Lancet Infectious Diseases 2, 33-42.

Misra, A., Mukerjee, R., Chaturvedi, U., 1996. Production of nitrite by dengue virus-induced cytotoxic factor. Clinical & Experimental Immunology 104, 406-411.

Misra, A., Mukerjee, R., Chaturvedi, U., 1998. Respiratory burst by dengue-virus-induced cytotoxic factor. Medical Principles and Practice 7, 251-260.

Mongkolsapaya, J., Dejnirattisai, W., Xu, X.-n., Vasanawathana, S., Tangthawornchaikul, N., Chairunsri, A., Sawasdivorn, S., Duangchinda, T., Dong, T., Rowland-Jones, S., 2003. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nature Medicine 9, 921-927.

Morales, I., Salje, H., Saha, S., Gurley, E.S., 2016. Seasonal distribution and climatic correlates of dengue disease in Dhaka, Bangladesh. The American Journal of Tropical Medicine and Hygiene 94, 1359-1361.

Murray, N.E., Quam, M.B., Wilder-Smith, A., 2013a. Epidemiology of dengue: past, present and future prospects. Clinical Epidemiology 5, 299-309.

Murray, N.E.A., Quam, M.B., Wilder-Smith, A., 2013b. Epidemiology of dengue: past, present and future prospects. Clinical Epidemiology 5, 299.

Mustafa, M.S., Rasotgi, V., Jain, S., Gupta, V., 2015. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Medical Journal Armed Forces India 71, 67-70.

Mutsuddy, P., Tahmina Jhora, S., Shamsuzzaman, A.K.M., Kaisar, S., Khan, M.N.A., 2019. Dengue situation in Bangladesh: An epidemiological shift in terms of morbidity and mortality. Canadian Journal of Infectious Diseases and Medical Microbiology 2019.

Nelson, M., Self, L., Pant, C., Usman, S., 1978. Diurnal periodicity of attraction to human bait of Aedes aegypti (Diptera: Culicidae) in Jakarta, Indonesia. Journal of Medical Entomology 14, 504-510.

Nguyet, M.N., Duong, T.H., Trung, V.T., Nguyen, T.H., Tran, C.N., Long, V.T., Dui le, T., Nguyen, H.L., Farrar, J.J., Holmes, E.C., Rabaa, M.A., Bryant, J.E., Nguyen, T.T., Nguyen, H.T., Nguyen, L.T., Pham, M.P., Nguyen, H.T., Luong, T.T., Wills, B., Nguyen, C.V., Wolbers, M., Simmons, C.P., 2013. Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. Proceedings of the National Academy of Sciences of the United States of America 110, 9072-9077.

Nimmannitya, S., Halstead, S.B., Cohen, S.N., Margiotta, M.R., 1969. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. American Journal of Tropical Medicine and Hygiene 18, 954-971.

Nisalak, A., Endy, T.P., Nimmannitya, S., Kalayanarooj, S., Thisayakorn, U., Scott, R.M., Burke, D.S., Hoke, C.H., Innis, B.L., Vaughn, D.W., 2003. Serotype-

specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. American Journal of Tropical Medicine and Hygiene 68, 191-202.

Normile, D., 2013. Surprising new dengue virus throws a spanner in disease control efforts. American Association for the Advancement of Science.

Nowak, T., Färber, P.M., Wengler, G., Wengler, G., 1989. Analyses of the terminal sequences of West Nile virus structural proteins and of the in vitro translation of these proteins allow the proposal of a complete scheme of the proteolytic cleavages involved in their synthesis. Virology 169, 365-376.

Nowak, T., Wengler, G., 1987. Analysis of disulfides present in the membrane proteins of the West Nile flavivirus. Virology 156, 127-137.

Ooi, E.-E., Goh, K.-T., Gubler, D.J., 2006. Dengue prevention and 35 years of vector control in Singapore. Emerging Infectious Diseases 12, 887.

Ooi, E.E., 2001. Changing pattern of dengue transmission in Singapore.

Osatomi, K., Fuke, I., Tsuru, D., Shiba, T., Sakaki, Y., Sumiyoshi, H., 1988. Nucleotide sequence of dengue type 3 virus genomic RNA encoding viral structural proteins. Virus Genes 2, 99-108.

Oviya, S., Kaviya, S., Udhaya, S., 2019. Dengue fever: Causes, complications, and vaccine strategies-A review. GSC Biological and Pharmaceutical Sciences 6, 16-23.

Perera, R., Kuhn, R.J., 2008. Structural proteomics of dengue virus. Current Opinion in Microbiology 11, 369-377.

Pervin, M., Sweety, A.A., Hossain, M.Z., Sharmin, R., Fatema, N., Rahman, M.A., Nehar, N., Yasmin, M., Khoda, M.M.E., Rahman, K.M., 2017. Sera-epidemiology of Dengue Virus Infection in Clinically Suspected Patients Attended in Dhaka Medical College Hospital During January to December 2016. Journal of Dhaka Medical College 26, 111-116.

Pervin, M., Tabassum, S., Ali, M., Mamun, K.Z., Islam, M., 2004. Clinical and Laboratory Observations Associated with the 2000 Dengue Outbreak in Dhaka, Bangladesh.

Pokharel, S.D.a.S., 2019. Bangladesh hit by worst dengue outbreak on record., CNN.

Polwiang, S., 2020. The time series seasonal patterns of dengue fever and associated weather variables in Bangkok (2003-2017). BMC Infectious Diseases 20, 1-10.

Powell, J.R., Tabachnick, W.J., 2013. History of domestication and spread of Aedes aegypti-A Review. Memórias do Instituto Oswaldo Cruz 108, 11-17.

Raheel, U., Faheem, M., Riaz, M.N., Kanwal, N., Javed, F., Qadri, I., 2011. Dengue fever in the Indian subcontinent: an overview. The Journal of Infection in Developing Countries 5, 239-247.

Rahman, M.L., Islam, M.M., Al Mamun, M., 2009. Commuter s Modal Choice: A Case Study of Savar Pourashava. Journal of Bangladesh Institute of Planners 2, 78-97.

Rajapakse, S., Rodrigo, C., Rajapakse, A., 2012. Treatment of dengue fever. Infection and Drug Resistance 5, 103.

Randolph, V.B., Winkler, G., Stollar, V., 1990. Acidotropic amines inhibit proteolytic processing of flavivirus prM protein. Virology 174, 450-458.

Ranjit, S., Kissoon, N., 2011. Dengue hemorrhagic fever and shock syndromes. Pediatric Critical Care Medicine 12, 90-100.

Reddy, M.N., Dungdung, R., Valliyott, L., Pilankatta, R., 2017. Occurrence of concurrent infections with multiple serotypes of dengue viruses during 2013–2015 in northern Kerala, India. PeerJ 5, e2970.

Rice, C., Strauss, E., Strauss, J., 1986. Structure of the flavivirus genome, p. 279-326. The Togaviridae and Flaviviridae doi 10, 978-971.

Rice, C.M., Lenches, E.M., Shin, S., Sheets, R., Strauss, J., 1985. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. Science 229, 726-733.

Rico-Hesse, R., 2003. Microevolution and virulence of dengue viruses. Advanced in Virus Reseasrch 59, 315-341.

Rico-Hesse, R., 2010. Dengue virus virulence and transmission determinants. Current Topics in Microbiology and Immunology 338, 45-55.

Rigau-Pérez, J.G., Clark, G.G., Gubler, D.J., Reiter, P., Sanders, E.J., Vorndam, A.V., 1998. Dengue and dengue haemorrhagic fever. The Lancet 352, 971-977.

Rodenhuis-Zybert, I.A., Wilschut, J., Smit, J.M., 2010. Dengue virus life cycle: viral and host factors modulating infectivity. Cellular and Molecular Life Sciences 67, 2773-2786.

Rothman, A.L., Ennis, F.A., 1999. Immunopathogenesis of dengue hemorrhagic fever. Virology 257, 1-6.

Russell, P.K., Brandt, W.E., Dalrymple, J.M., 1980. Chemical and antigenic structure of flaviviruses. The Togaviruses. Elsevier, 503-529.

Russell, P.K., Buescher, E.L., McCown, J.M., Ordoñez, J., 1966. Recovery of dengue viruses from patients during epidemics in Puerto Rico and East Pakistan. The American Journal of Tropical Medicine and Hygiene 15, 573-579.

Russell, P.K., Chiewsilp, D., Brandt, W.E., 1970. Immunoprecipitation analysis of soluble complement-fixing antigens of dengue viruses. The Journal of Immunology 105, 838-845.

Russell, P.K., Yuill, T.M., Nisalak, A., Udomsakdi, S., Gould, D.J., Winter, P.E., 1968. An insular outbreak of dengue hemorrhagic fever. II. Virologic and serologic studies. American Journal of Tropical Medicine and Hygiene 17, 600-608.

Sabin, A., 1950. The dengue group of viruses and its family relationships. Bacteriological Reviews 14, 225.

Sabin, A.B., 1952. Research on dengue during World War II. American journal of tropical Medicine and Hygiene 1, 30-50.

Salje, H., Rodríguez-Barraquer, I., Rainwater-Lovett, K., Nisalak, A., Thaisomboonsuk, B., Thomas, S.J., Fernandez, S., Jarman, R.G., Yoon, I.-K., Cummings, D.A., 2014. Variability in dengue titer estimates from plaque reduction neutralization tests poses a challenge to epidemiological studies and vaccine development. PLOS Neglected Tropical Diseases 8, e2952.

Salles, T.S., da Encarnação Sá-Guimarães, T., de Alvarenga, E.S.L., Guimarães-Ribeiro, V., de Meneses, M.D.F., de Castro-Salles, P.F., Dos Santos, C.R., do Amaral Melo, A.C., Soares, M.R., Ferreira, D.F., 2018. History, epidemiology and diagnostics of dengue in the American and Brazilian contexts: a review. Parasites & Vectors 11, 1-12.

Sellahewa, K.H., 2012. Pathogenesis of dengue haemorrhagic fever and its impact on case management. ISRN Infectious Diseases 2013.

Shapiro, D., Brandt, W.E., Russell, P.K., 1972. Change involving a viral membrane glycoprotein during morphogenesis of group B arboviruses. Virology 50, 906-911.

Sharmin, S., Viennet, E., Glass, K., Harley, D., 2015. The emergence of dengue in Bangladesh: epidemiology, challenges and future disease risk. Transactions of The Royal Society of Tropical Medicine and Hygiene 109, 619-627.

Shi, P.-Y., 2012. Molecular virology and control of flaviviruses. Caister Academic Press.

Shrivastava, A., Dash, P., Tripathi, N., Sahni, A., Gopalan, N., Rao, P.L., 2011. Evaluation of a commercial dengue NS1 enzyme-linked immunosorbent assay for early diagnosis of dengue infection. Indian Journal of Medical Microbiology 29, 51.

Siler, J.F., Hall, M.W., Hitchens, A.P., 1926. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. Philippine Journal of Science 29.

Smith., D.S., 2019. Dengue (The Aedes mosquito).

Smithburn, K., 1954. Antigenic relationships among certain arthropod-borne viruses as revealed by neutralization tests. The Journal of Immunology 72, 376-388.

Speight, G., Coia, G., Parker, M., Westaway, E., 1988. Gene mapping and positive identification of the non-structural proteins NS2A, NS2B, NS3, NS4B and NS5 of the flavivirus Kunjin and their cleavage sites. Journal of General Virology 69, 23-34.

Srikiatkhachorn, A., Krautrachue, A., Ratanaprakarn, W., Wongtapradit, L., Nithipanya, N., Kalayanarooj, S., Nisalak, A., Thomas, S.J., Gibbons, R.V., Mammen Jr, M.P., 2007. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. The Pediatric Infectious Disease Journal 26, 283-290.

Stephenson, J.R., 2005. Understanding dengue pathogenesis: implications for vaccine design. Bulletin of the World Health Organization 83, 308-314.

Stollar, V., Schlesinger, R.W., Stevens, T.M., 1967. Studies on the nature of dengue viruses: III. RNA synthesis in cells infected with type 2 dengue virus. Virology 33, 650-658.

Stollar, V., Stevens, T.M., Schlesinger, R.W., 1966. Studies on the nature of dengue viruses: II. Characterization of viral RNA and effects of inhibitors of RNA synthesis. Virology 30, 303-312.

Sumiyoshi, H., Mori, C., Fuke, I., Morita, K., Kuhara, S., Kondou, J., Kikuchi, Y., Nagamatu, H., Igarashi, A., 1987. Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. Virology 161, 497-510.

Svitkin, Y.V., Lyapustin, V.N., Lashkevich, V.A., Agol, V.I., 1984. Differences between translation products of tick-borne encephalitis virus RNA in cell-free

systems from Krebs-2 cells and rabbit reticulocytes: involvement of membranes in the processing of nascent precursors of flavivirus structural proteins. Virology 135, 536-541.

Tabassum, T., Taylor-Robinson, A.W., 2019. Dengue Serotypes in Bangladesh: Whole Genome Sequencing and Comparative Genomics Facilitates Pathogenesis and Epidemiology Studies and Informs Improved Disease Control. Microbiology and Infectious Disease 3, 1-5.

Takeda, H., Oya, A., Hashimoto, K., Yasuda, T., Yamada, M.-A., 1978. Association of virus specific replicative ribonucleic acid with nuclear membrane in chick embryo cells infected with Japanese encephalitis virus. Journal of General Virology 38, 281-291.

Thai, K.T., Nishiura, H., Hoang, P.L., Tran, N.T.T., Phan, G.T., Le, H.Q., Tran, B.Q., Van Nguyen, N., de Vries, P.J., 2011. Age-specificity of clinical dengue during primary and secondary infections. PLOS Neglected Tropical Diseases 5, e1180.

Thein, S., Aaskov, J., Myint, T.T., Shwe, T.N., Saw, T.T., Zaw, A., 1993. Changes in levels of anti-dengue virus IgG subclasses in patients with disease of varying severity. Journal of Medical Virology 40, 102-106.

Thein, S., Aung, M.M., Shwe, T.N., Aye, M., Zaw, A., Aye, K., Aye, K.M., Aaskov, J., 1997. Risk factors in dengue shock syndrome. American Journal of Tropical Medicine and Hygiene 56, 566-572.

Thomas, S.J., Endy, T.P., 2013. Current issues in dengue vaccination. Current Opinion in Infectious Diseases 26, 429-434.

Thomas, S.J., Yoon, I.-K., 2019. A review of Dengvaxia®: Development to deployment. Human Vaccines & Immunotherapeutics 15, 2295-2314.

Tjaden, N.B., Thomas, S.M., Fischer, D., Beierkuhnlein, C., 2013. Extrinsic Incubation Period of Dengue: Knowledge, Backlog, and Applications of Temperature Dependence. PLOS Neglected Tropical Diseases 7, e2207.

Tsai, J.-J., Chokephaibulkit, K., Chen, P.-C., Liu, L.-T., Hsiao, H.-M., Lo, Y.-C., Perng, G.C., 2013. Role of cognitive parameters in dengue hemorrhagic fever and dengue shock syndrome. Journal of Biomedical Science 20, 1-11.

Walsh, M., 2011. The mosquito and its ecology.

Wang, E., Ni, H., Xu, R., Barrett, A.D., Watowich, S.J., Gubler, D.J., Weaver, S.C., 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. Journal of Virology 74, 3227-3234.

Watts, D.M., Burke, D.S., Harrison, B.A., Whitmire, R.E., Nisalak, A., 1987. Effect of temperature on the vector efficiency of Aedes aegypti for dengue 2 virus. American Journal of Tropical Medicine and Hygiene 36, 143-152.

Wengler, G., 1989. Cell-associated West Nile flavivirus is covered with E+ pre-M protein heterodimers which are destroyed and reorganized by proteolytic cleavage during virus release. Journal of virology 63, 2521-2526.

Wengler, G., Wengler, G., Gross, H.J., 1978. Studies on virus-specific nucleic acids synthesized in vertebrate and mosquito cells infected with flaviviruses. Virology 89, 423-437.

Wengler, G., Wengler, G., Nowak, T., Wahn, K., 1987. Analysis of the influence of proteolytic cleavage on the structural organization of the surface of the West Nile flavivirus leads to the isolation of a protease-resistant E protein oligomer from the viral surface. Virology 160, 210-219.

Westaway, E., 1980. Replication of flaviviruses. The Togaviruses. Elsevier, 531-581.

Whitehorn, J., Farrar, J., 2010. Dengue. British Medical Bulletin 95, 161-173.

WHO, 1997. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. World Health Organization.

WHO, 2009. Dengue: guidelines for diagnosis, treatment, prevention and control. World Health Organization.

WHO, 2011. Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever.

WHO, 2012. Handbook for clinical management of dengue.

WHO, 2012-2020. Global strategy for dengue prevention and control 2012-2020.

WHO, 2014. Dengue and severe dengue. World Health Organization. Regional Office for the Eastern Mediterranean.

WHO, 2019. Dengue control.

WHO, 2020. Dengue & severe Dengue.

Wilder-Smith, A., Gubler, D.J., 2008. Geographic expansion of dengue: the impact of international travel. Medical Clinics of North America 92, 1377-1390, x.

Wilder-Smith, A., Ooi, E.-E., Horstick, O., Wills, B., 2019. Dengue. The Lancet 393, 350-363.

Wilder-Smith, A., Ooi, E.E., Vasudevan, S.G., Gubler, D.J., 2010. Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. Current Infectious Disease Reports 12, 157-164.

Wilder-Smith, A., Schwartz, E., 2005. Dengue in travelers. New England journal of medicine 353, 924-932.

Yang, K.D., Yeh, W.T., Yang, M.Y., Chen, R.F., Shaio, M.F., 2001. Antibody-dependent enhancement of heterotypic dengue infections involved in suppression of IFNγ production. Journal of Medical Virology 63, 150-157.

Ye, Y.H., Carrasco, A.M., Frentiu, F.D., Chenoweth, S.F., Beebe, N.W., van den Hurk, A.F., Simmons, C.P., O'Neill, S.L., McGraw, E.A., 2015. Wolbachia Reduces the Transmission Potential of Dengue-Infected Aedes aegypti. PLOS Neglected Tropical Diseases 9, e0003894.

Yung, C.-F., Lee, K.-S., Thein, T.-L., Tan, L.-K., Gan, V.C., Wong, J.G., Lye, D.C., Ng, L.-C., Leo, Y.-S., 2015. Dengue serotype-specific differences in clinical manifestation, laboratory parameters and risk of severe disease in adults, Singapore. The American Journal of Tropical Medicine and Hygiene 92, 999-1005.

Zhao, B., Mackow, E., Buckler-White, A., Markoff, L., Chanock, R.M., Lai, C.-J., Making, Y., 1986. Cloning full-length dengue type 4 viral DNA sequences: analysis of genes coding for structural proteins. Virology 155, 77-88.

Zivna, I., Green, S., Vaughn, D.W., Kalayanarooj, S., Stephens, H.A., Chandanayingyong, D., Nisalak, A., Ennis, F.A., Rothman, A.L., 2002. T cell responses to an HLA-B* 07-restricted epitope on the dengue NS3 protein correlate with disease severity. The Journal of Immunology 168, 5959-5965.