



Dengue Viral Infection in Children: Epidemiological, Nutritional and Genomic Analysis

August, 2025

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Dedicated

To

***My beloved late parents, my wife Dr. Puspanjali
Roy, dear daughter Bulbuli
&
All children who died from dengue viral
infections.***



Institute of Nutrition and Food Science

University of Dhaka

Dhaka, Bangladesh

PREFACE

This thesis entitled “**Dengue Viral Infection in Children: Epidemiological, Nutritional and Genomic Analysis**” is submitted to the Institute of Nutrition and Food Science, University of Dhaka, in partial fulfilment of the requirement for the degree of Master of Philosophy (MPhil). I am delighted that, for the first time in Bangladesh, dengue viral sequencing was carried out among children in this study. I hope this research will contribute to a better understanding of dengue infection and inspire further exploration in this area.

Date: August 19, 2025

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Certification by the Supervisor

The undersigned is pleased to certify that Kinkar Ghosh, MPhil student of Institute of Nutrition and Food Science, University of Dhaka, bearing the Reg. No-194, Session: 2020-2021 has successfully completed the thesis entitled **“Dengue Viral Infection in Children: Epidemiological, Nutritional and Genomic Analysis”** under my guidance and supervision. He has conducted the research with his full effort and sincerity from preparing the protocol up to finalization of reports. He has shown keep interest to know and learn the new things.

In order to appear in the final MPhil thesis examination for award, he is now permitted to submit the thesis report.

I wish his success in life.

Khaleda Islam

19.08.2025

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Acknowledgement

I thank the Almighty God for giving me the ability and opportunity to complete this thesis. Without 'His' blessing nothing is possible.

I express my special in debt of gratitude to my respected teacher and most sincere gratitude to my supervisor **Prof. Dr. Khaleda Islam**, Professor, Institute of Nutrition and Food Science, University of Dhaka, for her expert supervision, continuous supports and kind suggestions during the entire period of study and thesis writing. I am grateful to her that she gave me valuable time out of her busy schedule, otherwise I could never be completed this thesis in time.

I am appreciative of my family, especially my dear daughter **Bulbuli** and my better half, **Dr. Puspanjali Roy**, for their support and sacrifice.

I express my deep appreciation and full hearted thanks to all my teachers of Institute of Nutrition and Food Science, University of Dhaka. The time I spent in INFS together is the most enjoyable times in my life and also would like to give thanks to my course mate. Finally, I would like to thanks to guardian of the respondent (children) who cooperated with me in providing necessary information for the study during my data collection period.

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DECLARATION

I hereby declared that this thesis or any part of it has not been submitted elsewhere for the award of any Degree of Diploma or any other purpose.

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Abbreviation

ADE	: Antibody-Dependent Enhancement
BSL-2	: Biosafety Level
BSC	: Biological safety cabinet
CFR	: Case fatality rate
DENV	: Dengue Virus
DHF	: Dengue Hemorrhagic Fever
DSS	: Dengue Shock Syndrome
DORB	: Discharge of risk bond
DOR	: Discharge of risk
GMP	: Growth Monitoring Promotion
ICU	: Intensive Care Unit
MAS	: Macrophage activation syndrome
RDT	: Rapid Diagnostic Test
RT-PCR	: Reverse transcription polymerase chain reaction
RSV	: Respiratory Syncytial Virus
VTM	: Viral transport medium
WHO	: World Health Organization

Abstract

Dengue Viral Infection in Children: Epidemiological, Nutritional and Genomic Analysis

Background: The epidemiology and genomic characteristics of dengue viral infection in children have been poorly explored in Bangladesh. The study aimed to delineate the epidemiological, nutritional, and genomic characteristics of the dengue viral infection in Bangladeshi children. **Method:** A cross-sectional study was carried out in the in-patient department of Bangladesh Shishu Hospital and Institute from June 2023 to May 2024. Children of age <18 years admitted with presumptive positive dengue infection and presumptive negative dengue infection (NS1 and IgM negative) with features like dengue infection were enrolled in this study. A total of 722 presumptive dengue-positive children were included for epidemiological analysis, 349 children under the age of five for nutritional assessment; 104 dengue-positive children, and 50 dengue-negative children (from another group) for genomic analysis and concomitant viral infections respectively. MedCalc online tools and SPSS version 21 were used for genomic and statistical analysis. **Results:** Among 722 children, 600(83%) were from Dhaka City Corporation (494 and 106 were from North and South City Corporation respectively) and 122(17%) were from outside of Dhaka. The age group of <1 Year were 91(12%), 1- <5 Year 258(36%), 5- <10 Year 238(33%) and > 10 Year 135(19%). Out of all, fever was the compulsory presenting complaint, and vomiting 355(49%), abdominal pain 192(27%), loose motion 135(19%), cough 49(7%), headache 30(4%), reluctant to feed 23(3%), bleeding 18(2%), convulsion 16(2%) were present. Among all 516(71%) presented with warning signs and 206(29%) without warning signs; 82(16%) children with warning signs and 23(11%) without warning signs were admitted to the intensive care unit. Reinfection was in 39(5.4%), a comorbid condition in 79(10%) and shock was the predominant complication. Among 349 under-five children, 7 (2%) were overweight, 178(51%) normal, 153(44%) moderately underweight, and 11(3%) severely underweight. Real-Time PCR results reveal that out of 104 positive cases, 96(92%) were true positive and 8(8%) were false positive. Among 50 negative cases 17(34%) were false negative and 33(66%) true negative. Real-time PCR of nasopharyngeal swabs reveals that 12(12%) had Flu or Respiratory Syncytial Virus (RSV) in true positive cases(n=103) and 5 (19%) in true negative cases(n=26). Circulating serotypes were DEN2 87% and DEN3 13%. Genomic footprint shows that DEN-2-2023 in Bangladesh was most similar to 2018 and DEN-3-2023 most similar to 2017. **Conclusion:** In children, DEN2 was the most predominant serotype in 2023 among children which might be responsible for more severe diseases. Concomitant viral infection could be an upcoming concern for the dengue outbreak. Authorities should prioritize the feasibility of using Real-time PCR for dengue screening in the coming years and a laboratory network system like COVID-19 should be considered.

Keywords: Children, Dengue, Epidemiology, Serotype

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Abstract

Dengue Viral Infection in Children: Epidemiological, Nutritional and Genomic Analysis

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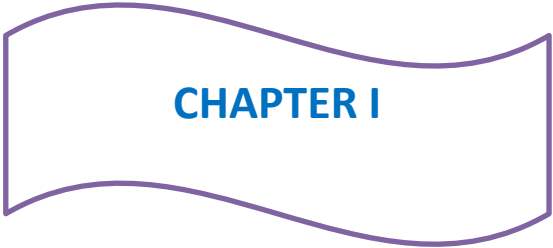
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CHAPTER I

1.1 Background of the study

1.2 Justification of the study

1.3 Research Question

1.4 Objectives of the study

CHAPTER I

1.1 Background of the study

Dengue fever is one of the most prevalent vector-borne diseases, primarily transmitted by *Aedes* mosquitoes, and it poses a significant health challenge worldwide. Dengue is the most common arboviral infection transmitted to humans by the bite of mosquito vectors *Aedes aegypti* and *Aedes albopictus* (1). It is caused by the Dengue virus (DENV), which exists in four serotypes (DENV-1, DENV-2, DENV-3, DENV-4). Globally, an estimated four billion people are at risk for Dengue virus (DENV) infection, with 96 million clinical cases. Although dengue affects individuals of all ages, children are particularly vulnerable, often presenting with more severe manifestations such as Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). The global burden of dengue has dramatically increased over the last few decades, with the World Health Organization (WHO) estimating that around 390 million people are infected annually, and a substantial proportion of these cases involve children. Dengue fever is the fastest-spreading mosquito-borne disease and has become a severe public health concern, particularly in tropical and subtropical nations like Bangladesh, a South Asian country with over 165 million populations.

Epidemiologically, the incidence of dengue in children varies across regions, with tropical and subtropical areas being the most affected due to favorable mosquito breeding conditions. Factors such as population density, urbanization, inadequate sanitation, and climate change significantly contribute to the widespread transmission of the virus. Children, particularly those under 10 years of age, are at an increased risk of developing severe forms of dengue due to their underdeveloped immune systems. Dengue infection affects the health of all age groups. However, the highest burden of dengue has been observed among children, adolescents, and young adults (2); in particular, the infection rate is highest among infants [WHO, 2009]. Moreover, dengue infections may progress to Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), which are associated with higher hospitalization and case fatality rates, particularly among children (3). Moreover, dengue serotypes DENV-1 to DENV-4, particularly in secondary infections, can lead to dengue severity due to Antibody-Dependent Enhancement (ADE).

Nutrition is crucial in determining the severity and outcome of dengue infections in children. Malnutrition, particularly protein-energy malnutrition, has been shown to affect immune function, potentially exacerbating the disease's severity. Inadequate nutrition may impair the body's ability to mount an effective immune response to the virus. Additionally, micronutrient deficiencies, such as those in vitamins A, C, and D, have been linked to heightened susceptibility to infections and complications in dengue. Research on the relationship between nutritional status and dengue severity in children is relatively limited, but existing studies suggest that proper nutrition might improve immune responses and reduce the likelihood of progression to more severe disease forms.

Considering the disease symptoms, many clinical features like fever, joint pain, body aches, rash, respiratory symptoms, gastrointestinal disorders, abnormal liver function tests, severe bleeding, organ impairment along with plasma leakage, dengue hemorrhagic fever (DHF), and life-threatening dengue shock syndrome (DSS) were most common in dengue patients. However, some symptoms, such as constipation and mouth sores, were rarely recorded (4).

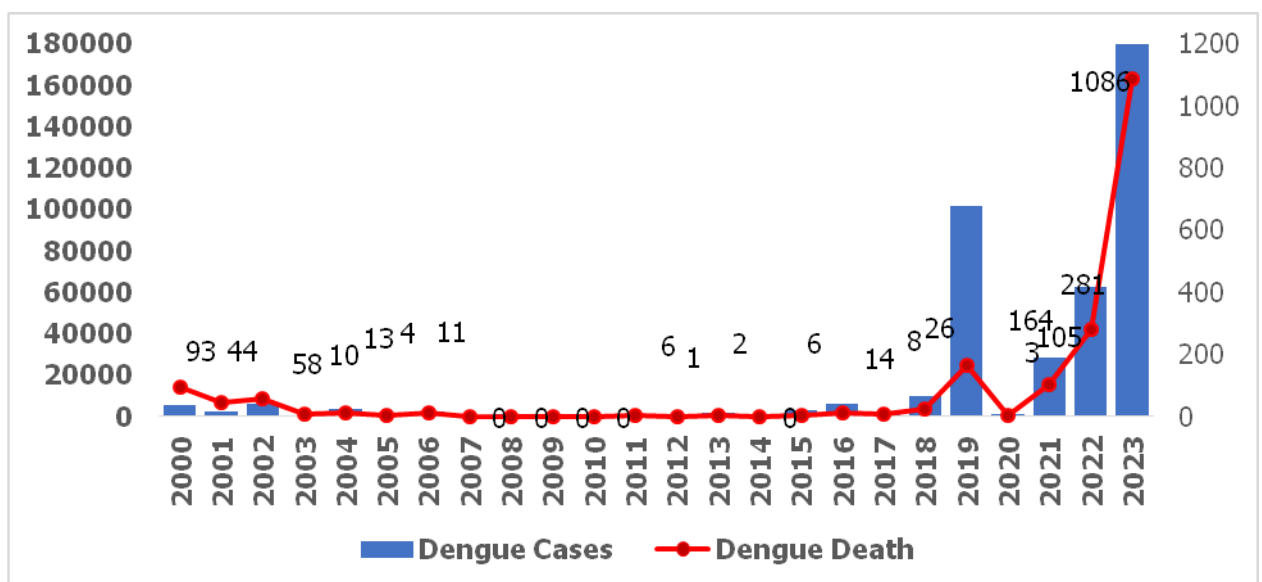
Additionally, RSV is a respiratory virus that is common among children. The resulting coinfection can deteriorate the health condition of the DENV-infected patients. A study found that a single diagnosis targeting DENV that is coinfecting with influenza A can increase the severity of diseases (5). However, the number of studies that focused on the coinfections among DENV, influenza, and RSV is limited. More studies are needed to establish the evidence that coinfection can happen and can be fatal to health.

On a genomic level, understanding the host-pathogen interaction in children with dengue is crucial for identifying biomarkers for early diagnosis and potential therapeutic targets. Recent advancements in genomics and bioinformatics have allowed researchers to explore the genetic predisposition of individuals to dengue infection. Genetic variants in immune-related genes such as those involved in cytokine production, immune signaling pathways, and cell signaling may influence the severity of the disease. Studies have shown that children with certain genetic markers might be more predisposed to severe dengue manifestations. This genomic analysis could lead to better prediction models for disease outcomes and personalized treatments for children affected by dengue.

1.2 Justification of the study

Bangladesh has been a dengue-endemic country since the first recorded outbreak in 2000. Over the years, in 2023, Bangladesh experienced the highest dengue outbreak in its history with 316,773 infections, 1652 mortality and 0.5% case fatality rate (CFR). If appropriate prevention measures are not implemented, insufficient outbreak readiness, weak healthcare infrastructure, and low community knowledge of dengue illness may result in public health catastrophes (6).

Yearly distribution of Dengue Infections in Bangladesh (2000-2023)



However, the pattern of DENV distribution is not unique. Several reports have documented that replication fitness, infection dynamics, and pathogenicity vary among DENV serotypes (7,8,9). For example, four dengue virus serotypes have been identified in Bangladesh [Mustafa et al., 2015], but the circulation pattern has changed over time. DENV-1 and DENV-2 were the dominating serotypes up to 2016 (10), followed by DENV-3, which was eventually responsible for the dengue outbreak in 2019 (11). In contrast, the preceding two years witnessed the prevalence of DENV-3 in 2021 and DENV-3, including DENV-4 in 2022 (12,13). Furthermore, various clades of DENV-2 caused diverse disease outcomes in children who had preexisting immunity to DENV-1 or DENV-3 (14). A few studies were conducted on the children to show the distribution of serotypes (15,16).

Those symptoms and demographic characteristics vary significantly between children and adults. For instance, children are less likely to experience pain symptoms from dengue infection (17,18,19). Furthermore, children infected with dengue virus in 2019 were older than those in the 2018 outbreak (20,21). This pattern of age distribution aligns with the 2000 outbreak, indicating a temporal shift of age involvement among Bangladeshi children (22, 23).

Furthermore, respiratory symptoms like cough, sore throat, and rhinorrhea have been observed in patients with dengue fever (24). In this respect, DENV circulation in endemic countries poses a diagnostic dilemma, especially during concurrent respiratory outbreaks. For children under five years, deaths from other lower respiratory infections continue to be of more significant concern because their death rates are more than ten times higher than those from tuberculosis (25). Additionally, in tropical countries like Bangladesh, the period of the highest indexes of DENV cases (June-August) coincides with seasonal outbreaks of respiratory viruses. As a result, there is a high possibility of getting coinfecting with other viruses, like influenza, that have the same seasonality (April-July).

Almost all the studies in Bangladesh's pediatric population documented the clinical manifestations of dengue in a single centre with a small number of samples (22,26,20). The severity of the disease has been linked to several clinical symptoms (27). Early detection and timely treatment of severe dengue can reduce dengue-associated mortality (28).

The need for a comprehensive approach to understanding dengue viral infection in children is critical due to the increasing global prevalence and the devastating impact it has on public health, particularly in developing countries. The lack of effective vaccines, coupled with the frequent outbreaks in regions where children are most vulnerable, highlights the importance of identifying risk factors for severe disease. Integrating epidemiological, nutritional, and genomic analyses provides a multidimensional perspective on the factors that contribute to disease severity in children, offering opportunities for improved prevention and treatment strategies.

Epidemiological studies can guide public health measures such as vector control and vaccination strategies, while nutritional interventions may offer an opportunity to improve disease outcomes in children. Furthermore, genomic studies could pave the way for precision medicine approaches, enabling the identification of genetic factors that predispose children to severe dengue, allowing for more targeted interventions. The findings from these analyses

could inform public health policies, contribute to the development of vaccines, and guide nutritional and healthcare strategies to reduce the burden of dengue on children.

1.3 Research Question

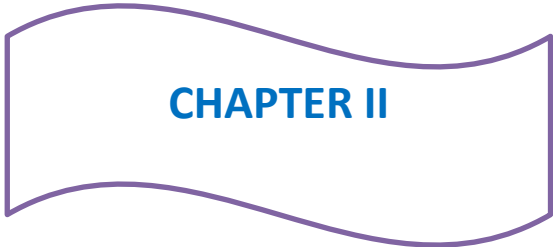
What are the epidemiological, nutritional and genomic characteristics of dengue viral infection in children?

1.4 Objectives of the study

General Objective: The study was designed to evaluate the epidemiological, nutritional, and genomic characteristics of dengue viral infection in children at a tertiary level hospital in Bangladesh.

Specific Objectives:

- To observe the clinico-epidemiological features of dengue virus infection in children
- To evaluate children's nutritional status in relation to dengue virus infection
- To identify the dengue virus circulating serotypes in children during the 2023 outbreak
- To assess coinfection with respiratory viruses of dengue infection in children (RSV and Influenza)
- To compare the diagnostic performance of rapid antigen tests to RT-PCR in dengue virus infection diagnosis



CHAPTER II

Literature Review

CHAPTER-II

Literature review

Epidemiological Overview of Dengue in Children in Bangladesh

In Bangladesh, dengue has been endemic for decades, with a significant increase in the number of cases reported in recent years. According to the World Health Organization (WHO), the country has seen a steady rise in dengue transmission, particularly during the monsoon season when mosquito breeding conditions are optimal. The first major outbreak occurred in 2000, and subsequent years have seen increasing outbreaks, especially in urban areas such as Dhaka, Chittagong, and Sylhet (4).

A study by Rahman et al. (2018) found that children under the age of 15 accounted for nearly 40% of all reported dengue cases in Bangladesh. The incidence rate is particularly high among children under 10 years of age, with a marked increase in the occurrence of severe dengue. Several studies suggest that children are at greater risk of severe forms due to their developing immune systems, making them more vulnerable to complications such as hemorrhagic manifestations, vascular leakage, and shock (29). Additionally, a study conducted in Dhaka reported that 60% of hospitalized dengue patients under the age of 12 required intensive care for DHF or DSS, highlighting the severe burden dengue places on children (30).

Nutritional Status and Its Impact on Dengue in Children

Several studies conducted in Bangladesh have examined the role of nutritional status in the immune response to dengue. Poor nutritional status, including deficiencies in vitamins A, C, and D, has been associated with impaired immune function, making children more susceptible to severe disease outcomes. A study by Khan et al. (2019) found that children with low serum levels of vitamin A were more likely to develop severe dengue symptoms and were at higher risk for complications such as hemorrhage and organ failure.

A study by Hossain et al. (2017) demonstrated that micronutrient deficiencies, particularly zinc and vitamin C, were common in children with dengue in Bangladesh. These deficiencies were correlated with a greater likelihood of developing severe dengue, as these nutrients are critical

in supporting immune responses. Zinc, in particular, is known to play an essential role in T-cell function and immune regulation, which is vital for combating viral infections like dengue (Hossain et al., 2017). Improving the nutritional status of children in Bangladesh through targeted interventions, such as micronutrient supplementation, could therefore help reduce the severity of dengue infections.

Malnutrition also complicates the management of dengue, as it can affect fluid balance and immune response, making it harder to control the disease. Children with inadequate nutrition often require more intensive treatment and are at a higher risk for complications, thus placing additional strain on healthcare resources in Bangladesh (29). Nutritional interventions, such as protein-rich diets and micronutrient supplementation, have been suggested as a potential strategy for improving the outcomes of dengue in children

Genomic Insights into Dengue Infection in Children in Bangladesh

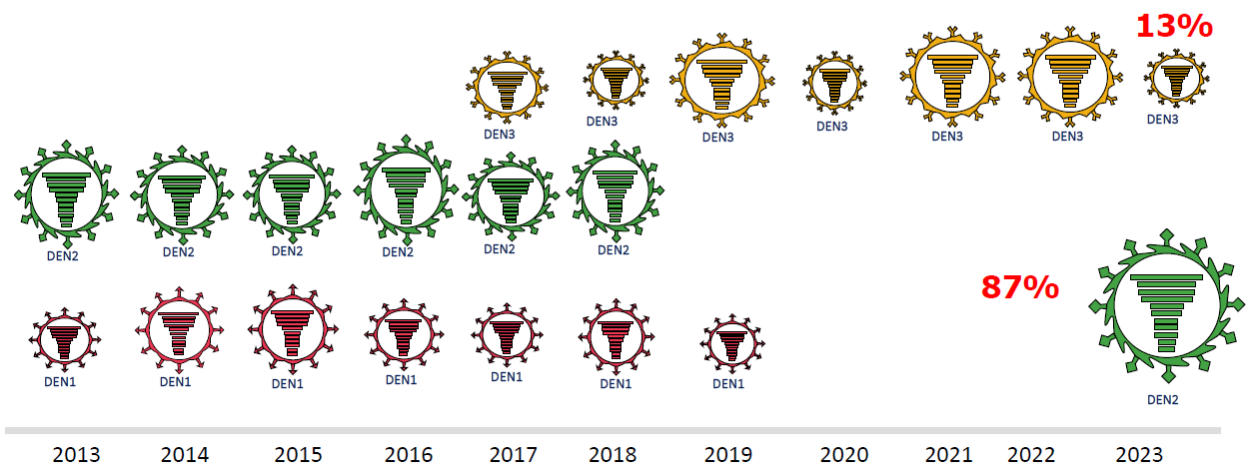
The genetic factors that influence the severity of dengue infections in children are an emerging area of research in Bangladesh. While much of the focus has been on the virus itself, understanding the host's genetic predisposition to severe disease is crucial in predicting and preventing adverse outcomes.

Circulating Dengue Serotypes in Bangladesh

The serotypes of dengue virus that circulate in Bangladesh have been studied extensively in recent years. Studies have shown that all four dengue serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) have been detected in various regions of the country, with variations in their distribution over time.

The co-circulation of multiple dengue serotypes is a significant feature of dengue transmission in Bangladesh. Early studies indicated that DENV-2 was the predominant serotype during the initial years of the 21st century (2000–2005) (Rahman et al., 2006). However, more recent studies have shown that DENV-3 has become more prevalent in recent outbreaks, particularly in Dhaka, the capital, which has witnessed larger outbreaks of dengue since 2010 (31). A study by Sultana et al. (2015) indicated that DENV-3 was responsible for the majority of the cases in the 2014 outbreak, contributing to higher rates of severe disease.

Circulating serotypes/genotypes of Dengue Virus Bangladesh: 2013-2022



The burden of dengue infection in children in Bangladesh is increasing, and the interplay between epidemiological, nutritional, and genomic factors complicates disease management and prevention. Epidemiological studies highlight the growing incidence of dengue in urban areas, while malnutrition exacerbates the severity of the disease. Additionally, genomic research suggests that genetic predispositions in children may influence their susceptibility to severe dengue manifestations.

The circulating serotypes and genotypes of dengue virus in Bangladesh exhibit significant variation, which is crucial for understanding the dynamics of dengue transmission and the factors that influence disease severity, particularly in children. The predominance of certain serotypes in different outbreaks and the genetic diversity of circulating strains underscore the complexity of managing dengue in Bangladesh.



CHAPTER III

Material and Method

- 3.1 Study Design**
- 3.2 Study Area**
- 3.3 Study Period**
- 3.4 Study Population**
- 3.5 Selection Criteria**
- 3.6 Sample Size**
- 3.7 Methodological Diagram**
- 3.8 Data collection instrument**
- 3.9 Data collection technique**
- 3.10 Specimens' collection**
- 3.11 Laboratory procedure**
- 3.12 Data Analysis**
- 3.13 Ethical clearance**
- 3.14 Limitations of the study**
- 3.15 The strengths of the study**

CHAPTER-III

METHODOLOGY

Methodology is a crucial part which makes the study scientific and provides a way to reach the objective of research. This chapter presents an outline of approach and methodology of the study.

3.1 Study Design

This was a cross-sectional study to assess the epidemiological, nutritional and genomic characteristics of dengue viral infection in children from a tertiary-level hospital in Bangladesh.

3.2 Study Area

Participants were selected from the in-patient department at Bangladesh Shishu Hospital & Institute, located in the capital city of Dhaka. The average occupancy rate in 2022 was about 90%. By 2017, the capacity had risen to 650 beds and as of 2020, it was the largest children's hospital in Bangladesh.

3.3 Study Period

The total study lasted for a period of 12 months from July 2023 to June,2024. To complete the study in time, a work schedule was prepared depending on different tasks attached at annexure. The first 3 month were spent for literature review, topic selection, development of protocol, and approval of the protocol by the board. The subsequent 3 months were spent for questionnaire development and pre-testing, data collection, compilation and analysis, report writing, printing, and submission of the report. Literature review was simultaneously going on till the final report was writing.

3.4 Study Population

In this study, participants were selected from the in-patient department at Bangladesh Shishu Hospital & Institute. The selected participants age below 18 years, either sex was requested to participate in the study. Children who were admitted to Bangladesh Shishu Hospital & Institute with presenting dengue-like symptoms and NS1 or IgM-positive during the data collection period (June,2023-August,2023).

3.5 Selection Criteria

Inclusion Criteria

1. Participants aged 1-15 years
2. Both male and female
3. Admitted children who were positive for rapid test (NS1-Ag, IgM, and IgG)
4. Agree to provide biological samples
5. Participants with informed consent of parents

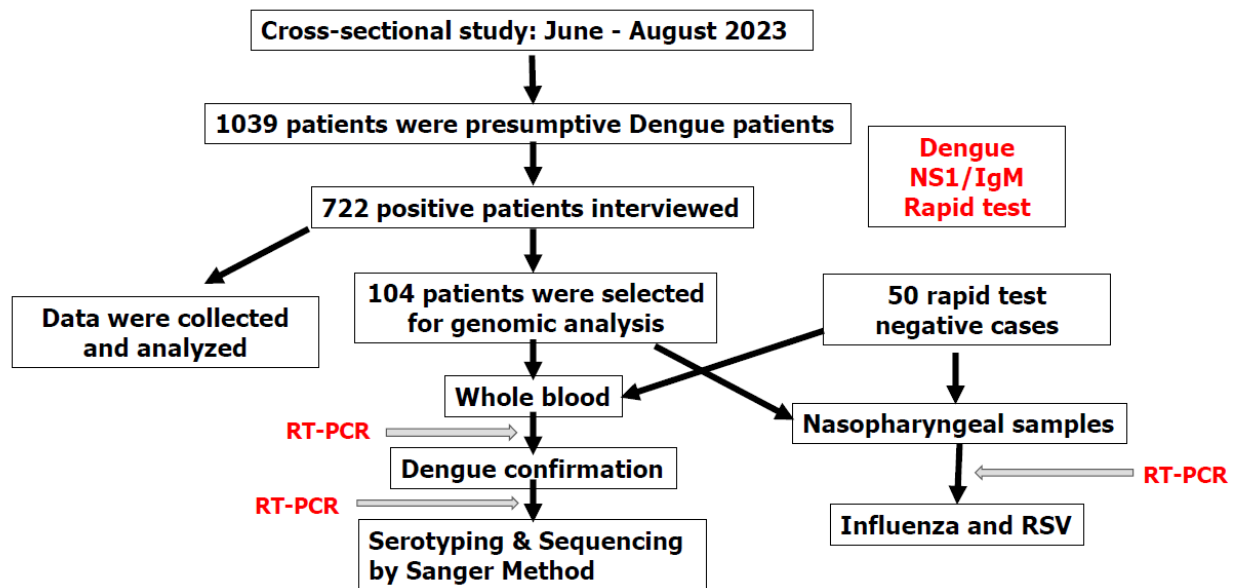
Exclusion Criteria

1. Patients who were diagnosed with immunosuppressive diseases
2. Patients who were unwilling to participate

3.6 Sample Size

The study primarily selected 1069 participants who were admitted during the data collection period. Among them, A total 722 participants were enrolled in this study which age group were 1 year to >10 years according to inclusion criteria. All participants were included for epidemiological analysis. Due to unsupported setting (most of the participants were hemodynamically unstable and restless so did not approach and parents did not allow for height measurement or anthropometric measurement which stated in limitation of study) only weight for age was considered for age up to five years (59 months full) which were 349 among 722. Out of 722 selected participants, 104 cases were purposively selected for molecular testing. Additionally, 50 dengue NS1 negative participants were enrolled purposively and were also included in the study for molecular testing.

3.7 Methodological Diagram



3.8 Data collection instrument

A structured pre-tested questionnaire and observational checklist were used for data collection. The questionnaire and checklist were pilot-tested before rollout. Laboratory accessories were used for hematological samples. Bed side dengue data sheet and patients' admission and detailed history form were used to record clinical manifestations, warning signs and outcome.

Case definition: Dengue cases were confirmed by physician according to National Dengue Clinical Case Management Guideline 2022(Revised).

For Nutritional Assessment: Stadiometer, Bathroom Scale, GMP Card, measuring tape were used for nutritional data.

3.9 Data collection technique

With the guardian's consent, a validated self-structured questionnaire was used for data collection. A qualified phlebotomist collected hematological samples while maintaining aseptic precautions, and the sample was stored at laboratory temperature before sending to the laboratory. Face-to-face interviews were used to collect the demographic data while keeping patient privacy and health settings in mind.

3.10 Specimens' collection

We collected 2ml blood for those up to 1 year of age, 3ml blood for up to 3 years of age, and 5ml blood for the rest of the patients. Along with blood, a nasopharyngeal swab specimen was taken in 5 ml VTM for both detection of dengue and other concomitant virus.

3.11 Laboratory procedure

3.11.1 Sample Processing

After rapid screening, collected blood samples were sent to the Virology Laboratory, icddr, and processed in a dedicated BSL-2 laboratory with BSL-3 practices under a certified Class II biological safety cabinet (BSC). Once a clinical specimen has been treated with lysis buffer for RNA extraction, the samples can be moved to a less restrictive environment to complete the RNA extraction and Real-time RT-PCR.

3.11.2 Nucleic acid extraction

Viral RNA was extracted and purified from blood samples according to the proper laboratory manual. QIA amp Viral RNA Mini Kit (Qiagen), serotype-specific consensus primers and probes were used for extraction.

3.11.3 Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

Dengue virus nucleic acid was detected by real-time RT-PCR. Briefly, the screening and serotyping were performed using DENV and their serotype-specific consensus primers and probes to detect any dengue virus-positive RNA [Santiago et al., 2013]. The amplification was carried out in a 20 µl final reaction volume comprising the Applied Biosystems™ TaqPath™ buffer of 5.0 µl, forward and reverse primers of 0.5 µl each (final concentration 0.5 µM), probe of 0.5 µl (final concentration 0.25 µM), 8.5 µl of nuclease-free H₂O, and 5 µl of RNA template. The thermocycling program for both DENV screening and serotyping is as follows: Pre-incubation at 25°C for 2 min, RT step at 53°C for 10 min, followed by 95°C for 2 min, and 45 cycles of denaturation at 95°C for 15 seconds, followed by annealing at 60°C for 1 minute. A sample generating a threshold cycle (CT) value ≤ 40 was considered positive. $Ct < 40$ was evaluated as positive, and Ct value > 40 was negative.

3.11.4 Sequencing: Partial genome sequencing was performed by Sanger method. Total 110 positive dengue cases confirm by RT-PCR were enrolled for sequencing.

3.12 Data Analysis

MedCalc online tools and SPSS version 21 were used for this investigation for all statistical analyses. Categorical variables were compared using χ^2 tests. Figures (Bar Graphs, Pie charts, etc.) were developed using MS Excel.

3.13 Ethical clearance

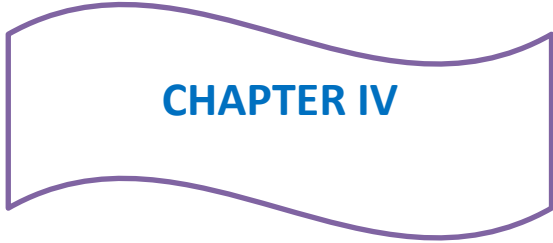
The institutional review board (IRB) of Bangladesh Shishu Hospital and Institute (BSH&I) has given their approval for the study.

3.14 Limitations of the study

- Due to funding limitations, real-time RT-PCR could not be performed for each of the positive cases and only 50 cases enrolled from negative group.
- Height measurement could not be taken in unsupported setting.

3.15 The strengths of the study

- The strengths of the study were that it highlighted several critical insights into the epidemiology and clinical presentation of dengue virus infection in the pediatric population of Bangladesh that had been explored less from Bangladesh's perspective yet.
- The identification of dengue and influenza virus coinfection through RTPCR indicates a need for comprehensive viral surveillance to better understand coinfection dynamics and their impact on disease severity and outcomes.



CHAPTER IV

- Section 4.1: Demographic characteristics**
- Section 4.2: Clinical characteristics**
- Section 4.3: Nutritional Characteristic**
- Section 4.4: Impact of hospital stay on fever duration, antibiotic use, and disease outcomes**
- Section 4.5: Coinfection status of the children**
- Section 4.6: Screening and serotyping of Dengue virus using RT-PCR**
- Section 4.7: Comparison of the diagnostic performance of rapid antigen tests to RT-PCR**

CHAPTER-IV

RESULTS

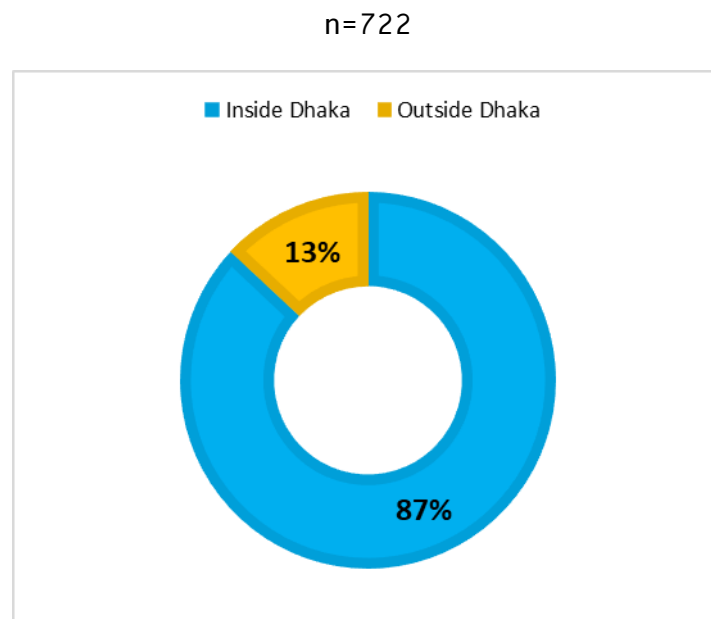
The study was carried out among 722 Bangladeshi Dengue-positive children aged 1-15 years. Data were collected from the Department of Clinical Pathology of Bangladesh Shishu Hospital & Institute, Dhaka. This study focused on exploring the epidemiological, nutritional and genomic scenario of pediatric dengue virus infection from a tertiary-level hospital in Bangladesh. The study findings are presented in this chapter. Outputs are presented both in tabular and graphical form.

Section 4.1: Demographic characteristics

4.1.1 Distribution of participants by location (both inside and outside of Dhaka)

The study revealed that among 722 children in this study, most (87%, 600) participants came from inside Dhaka and the rest from outside Dhaka. (Figure-1)

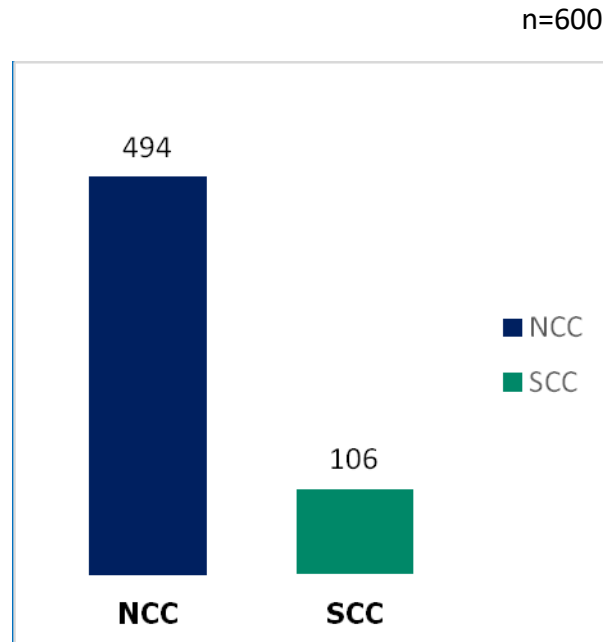
Figure: 1 Distribution of the children by location (both inside and outside of Dhaka)



4.1.2 Distribution of participants by location (inside Dhaka city)

In Dhaka city, 82% (n= 494) were from Dhaka North City Corporation, and 18% (n = 106) from the Dhaka South City corporation. (Figure-2)

Figure: 2 Distribution of the children by location (inside Dhaka city)



4.1.3 Distribution of Dengue Viral Infection by district

According to the study findings most (n= 624) of the dengue patient came from inside the Dhaka city. (Table 1)

Table 1 Distribution of Dengue Viral Infection by district

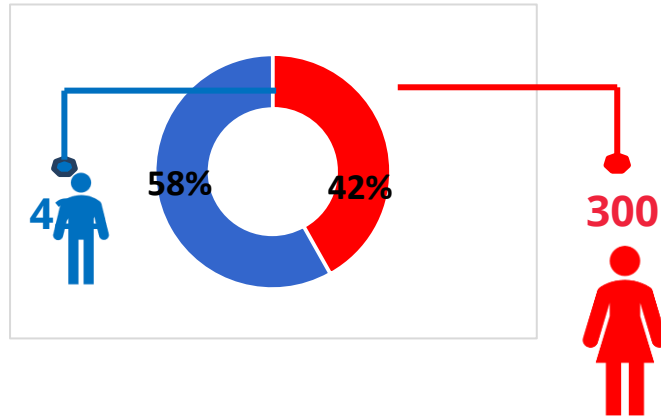
District	Frequency
Dhaka	624
Madaripur	8
Lakshmipur	7
Gazipur	6
Narayanganj	6
Shariatpur	6
Barisal	5
Bhola	5
Gopalganj	5
Narsingdi	5
Sirajganj	5
Manikganj	4
Chandpur	3
Comilla	3
Faridpur	3

4.1.4 Gender distribution of children

Figure 3 shows that among 722 children in this study, 58% (422) were male and 42% (300) were female. (Figure-3)

Figure: 3 Distribution of the children by gender

n=722

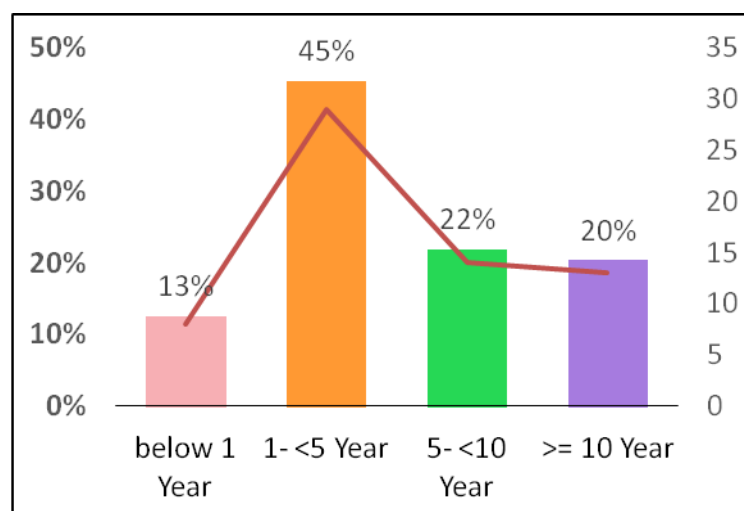


4.1.5 Distribution of participants by age (true outside of Dhaka)

According to the findings, almost half were between 1-<5 years old followed by 22%, 5-<10 years, 20% from >10 years of age and 13% of participants were from <1 year. (Figure-4)

Figure: 4 Distribution of the children by gender

n=64

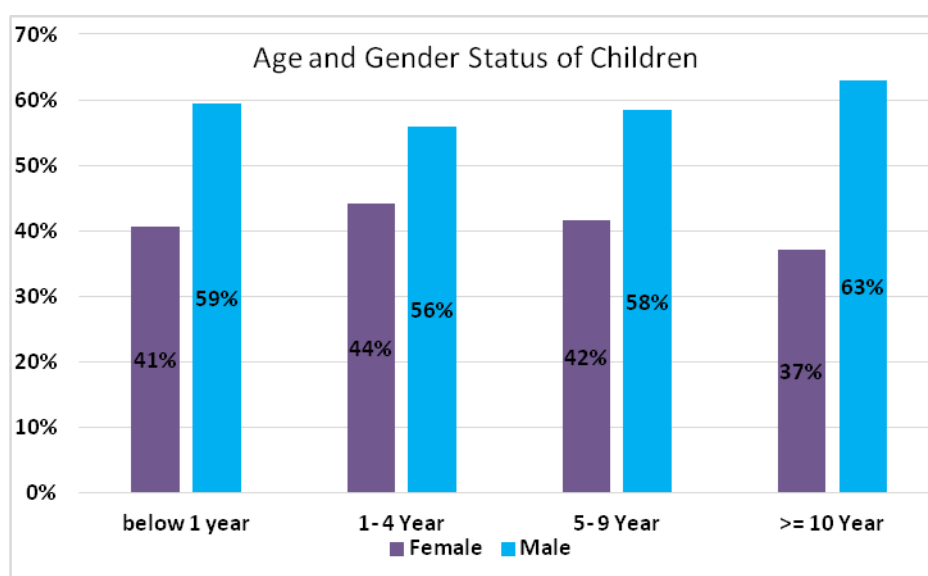


4.1.6 Age and Sex Distribution of Children

According to the study findings, the percentage of males was more than fifty percent among all dengue-positive participants in all age groups. Males over the age of 10 years represented 63%. On the other hand, 44% of females were from the age group 1-4 years. (Figure-5)

Figure: 5 Distribution of the children by age and sex

n=722



Section 4.2: Clinical characteristics

4.2.1 Distribution of participants by clinical characteristics

Concerning clinical symptoms study found, that all the participants had a fever (100%), followed by vomiting (49%), abdominal pain (27%), loose motion (19%), and cough (7%). Moreover, 4% of participants showed headache, 3% were reluctant to eat, 2% showed convulsions, bleeding, body aches, and rash, and 1% showed breathing difficulty. (Table 2)

Table 2: Distribution of the children by clinical characteristics

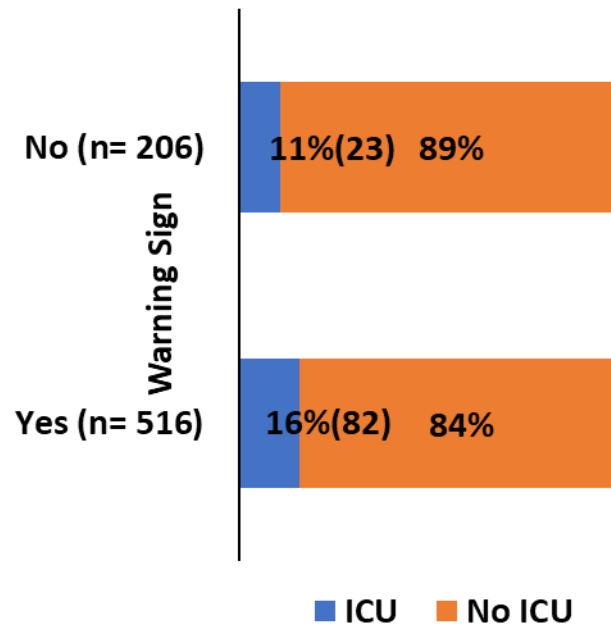
n=722

Symptoms	Frequency	Percentage (%)
Fever	722	100
Vomiting	355	49
Abdominal pain	192	27
Loose motion	135	19
Cough	49	7
Headache	30	4
Reluctant to feed	23	3
Bleeding	18	2
Convulsion	16	2
Body ache	14	2
Rash	14	2
Breathing difficulty	5	1

4.2.2 Distribution of participants by warning sign

The study reveals that among all the cases, 71% (n=516) had the warning sign, and 29%(n=206) of participants did not show any warning signs. Moreover, only 11% required ICU who had no warning signs, and on the other hand, 16% required ICU with warning signs. (Figure 6)

Figure 6: Distribution of the children by warning sign



4.2.3 Monthly Distribution of participants by cases (deaths)

Regarding the monthly distribution, the number of cases in June was only 51 (death 1), while the cases in July and August were 291 (death 8) and 380 (death 8), respectively. (Table 3)

Table 3: Distribution of monthly cases (deaths)

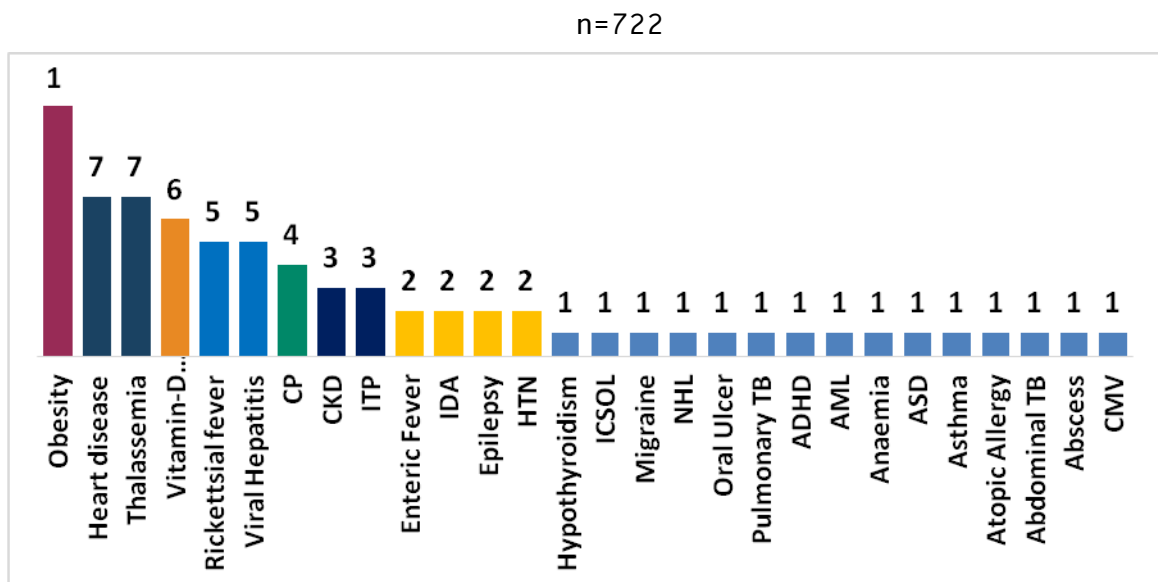
n=722

Month	Frequency of cases (deaths)	Percentage (%) of cases (deaths)
June	51 (1)	7 (6)
July	291 (8)	40 (47)
August	380 (8)	53 (47)

4.2.4 Distribution of participants by comorbidities

The study found that 10% (78 out of 722) patients had comorbid conditions, of which 11 had obesity, 7 had heart disease and thalassemia, 6 for vitamin D-deficiency, 5 for rickettsial fever and viral hepatitis, 4 for CP, 3 for CKD and IPD, 2 for enteric IDA, Epilepsy, HTN, and 1 for other diseases. (Figure 7)

Figure 7: Distribution of the children by warning sign



4.2.5 Distribution of participants by complications

Regarding major complications, shock was observed in 17% of patients, followed by macrophage activation syndrome (MAS) in 3.2%, pneumonia in 2.6%, and myocarditis in 1.8%. The remaining complications occurred in 1% or less of patients (Figure 8a). Among those with shock, 96% experienced compensated shock, and 4% had decompensated shock. (Figure 8b)

Figure 8a: Distribution of the children by complications

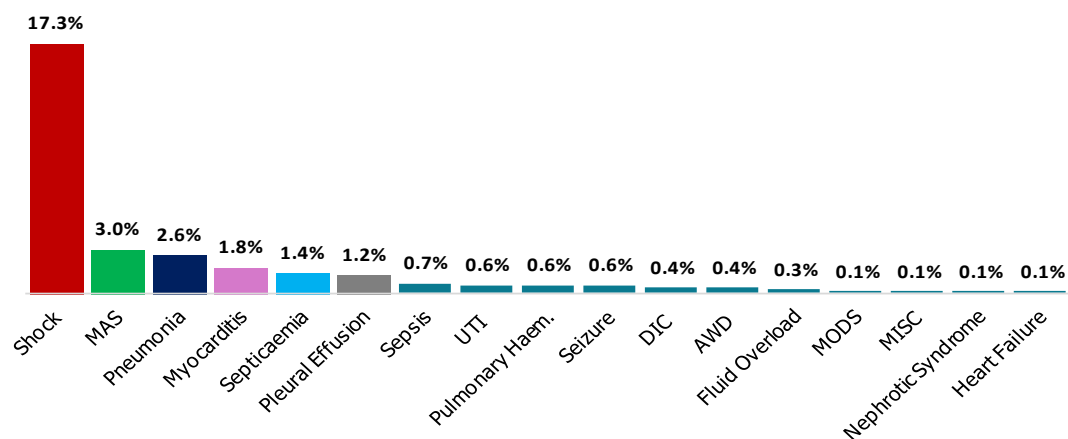
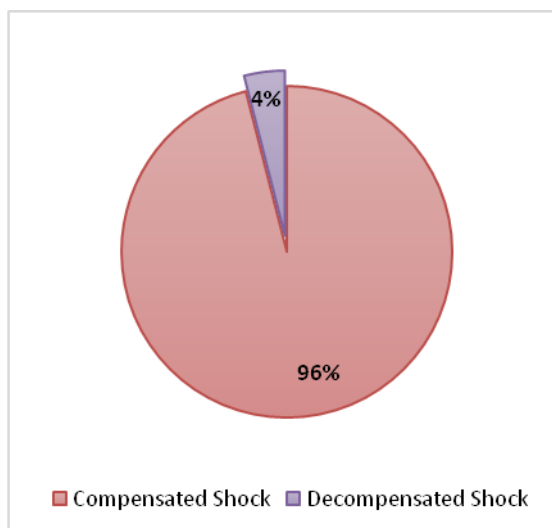


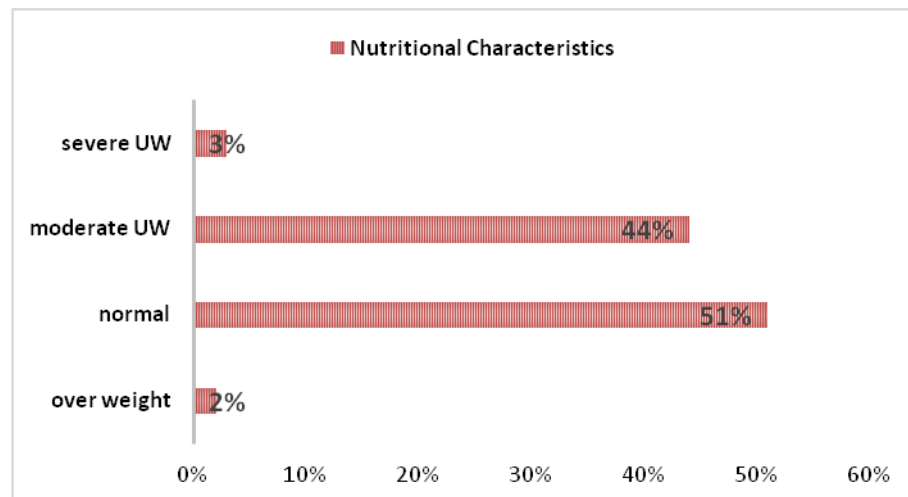
Figure 8b Distribution of the children by shock



Section 4.3: Nutritional Characteristic

Among 722, Only 349 respondents up to age 5 years were enrolled. The study revealed half of them found normal and 44% were in moderate underweight. Only 3% of them had severe underweight whereas 2% had overweight. (Figure 9)

Figure 9 Distribution of Nutritional status



Section 4.4: Impact of hospital stay on fever duration, antibiotic use, and disease outcomes

4.4.1 Impact of hospital stay on fever duration

According to study findings during the dengue season, 71% of the patients stayed in the hospital for 3-7 days; however, the median hospital stay was 9.5 days. Interestingly, after ten days, the number of patients decreased, and only a few percent took more than 15 days to recover from the infections. It was observed that patients who were diagnosed within 3-6 days of fever onset required only 5 days to stay in hospital. However, those who were tested after more than 6 days of symptom onset required more than 5 days to stay in the hospital. (Figure 10a and 10b)

Figure 10a Impact of hospital stay on Fever Duration

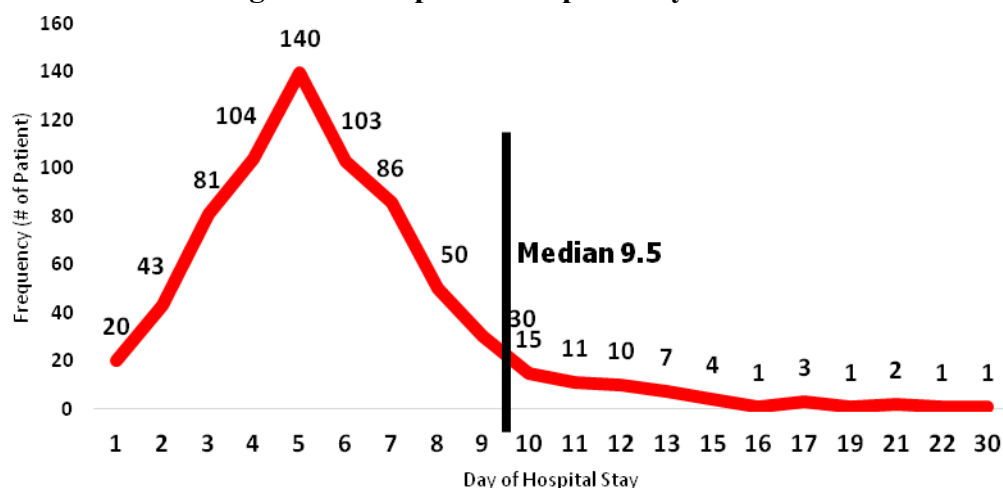
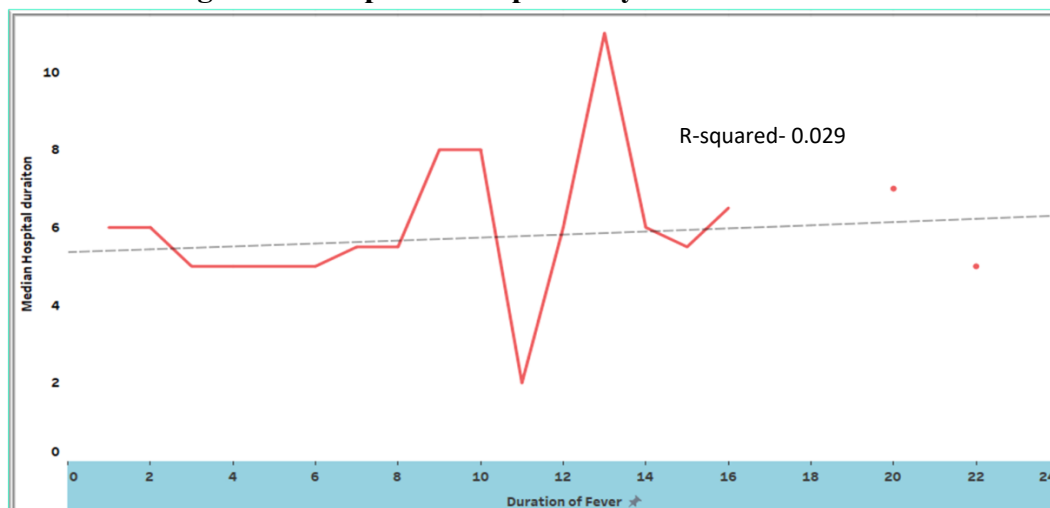


Figure 10b Impact of hospital stay on Fever Duration



4.4.2 Distribution of the patient by antibiotic used

During hospital stay, 82% (n=549) patients received at least one antibiotic, 16% (n=111) two antibiotics, and 1% with 3 and 4 different types of antibiotics. (Table 4)

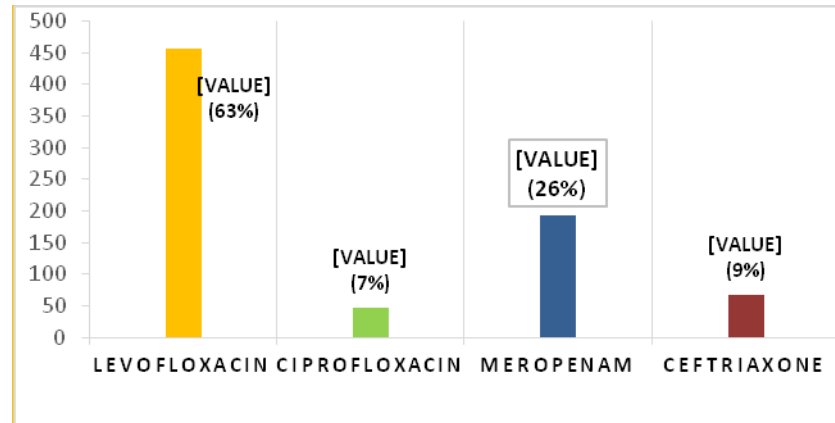
Table 4: Distribution of the patient by antibiotic use

Antibiotic used	Frequency of the patient	Percentage of the patient
Only 1	549	82
2 different	111	16
3 different	7	1
4 different	3	1

4.4.3 Distribution of common antibiotic used

Among the administrated antibiotics, the highest percentage was levofloxacin (63), followed by meropenem (26), ceftriaxone (9), and ciprofloxacin (7) respectively. (Figure 11).

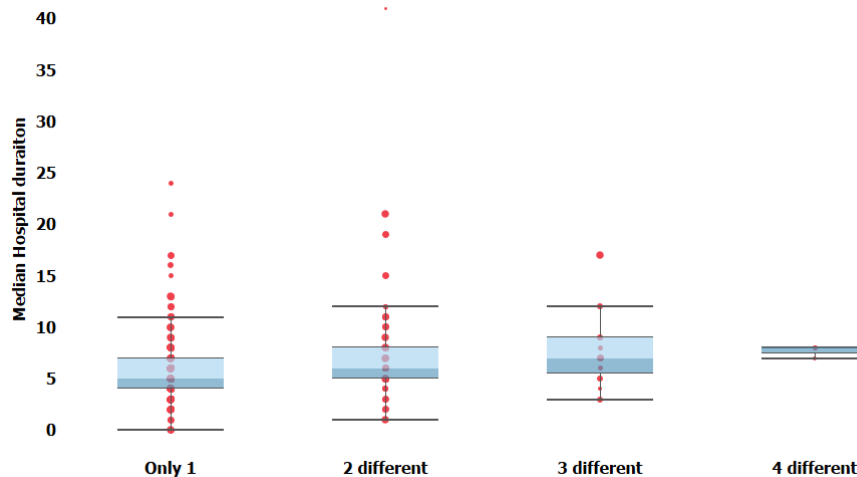
Figure 11 Distribution of common antibiotic used



4.4.4 Impact of hospital stay on antibiotic use

The length of the hospital stay was positively correlated with the use of multiple antibiotics. Patients with an average stay of 5 days took only one antibiotic, whereas those with an average stay of 6, 7, and 8 days used 2-4 different types of antibiotics. (Figure 12)

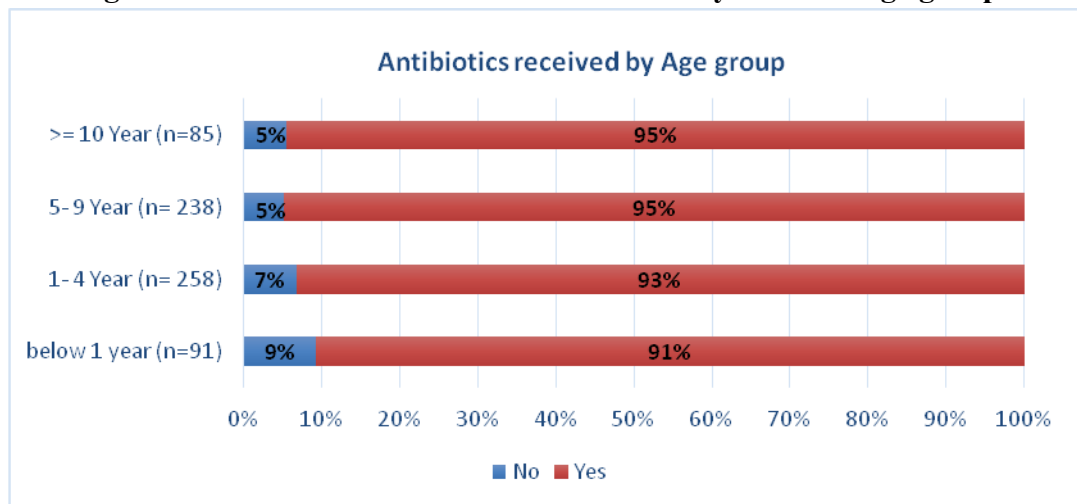
Figure 12 Impact of hospital stay on antibiotic use



4.4.5 Distribution of antibiotics received by different age groups

According to the study findings, 670 children out of 722 received antibiotics. More than ninety percent of all age groups received antibiotics. (Figure 13)

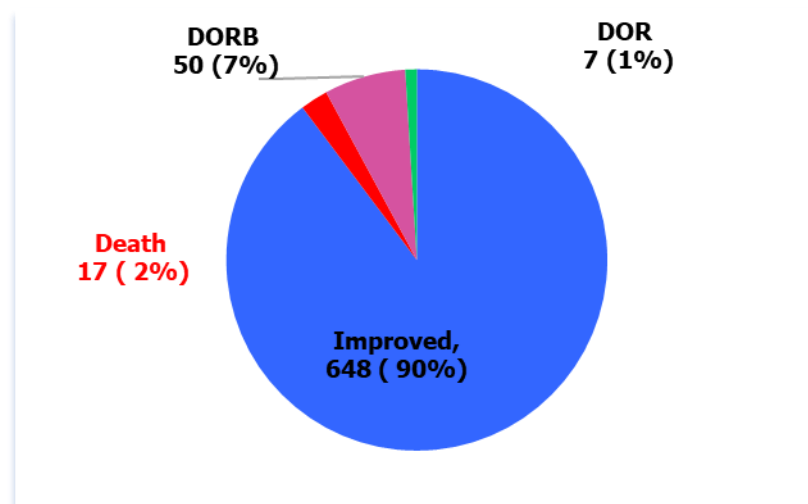
Figure 13 Distribution of antibiotics received by different age groups



4.4.6 Impact of hospital stay on patient outcomes

Regarding the outcome of dengue infection, it was observed that 90% (n = 648) of patients recovered from the disease, 7% were in DORB (Discharge of risk bond) condition, 2% of patients died and 1% were in DOR (Discharge of risk) conditions (Figure 14).

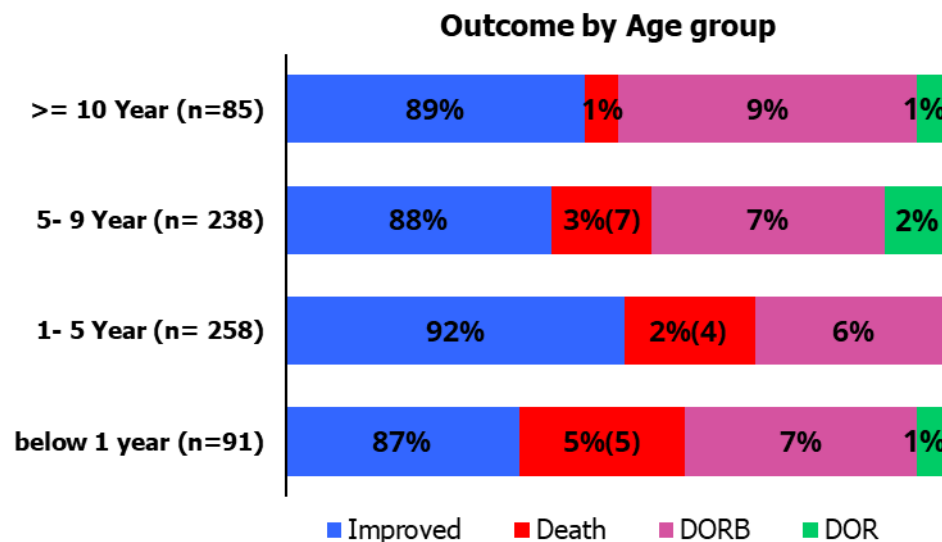
Figure 14 Impact of hospital stay on antibiotic use



4.4.7 Distribution of disease outcome by age

The study reveals that most (92%) of the children improved in the age group 1-5 years; in the other age groups, almost ninety percent of children improved. On the other hand, the highest percentage of deaths occurred in children under 1 year. (Figure 15)

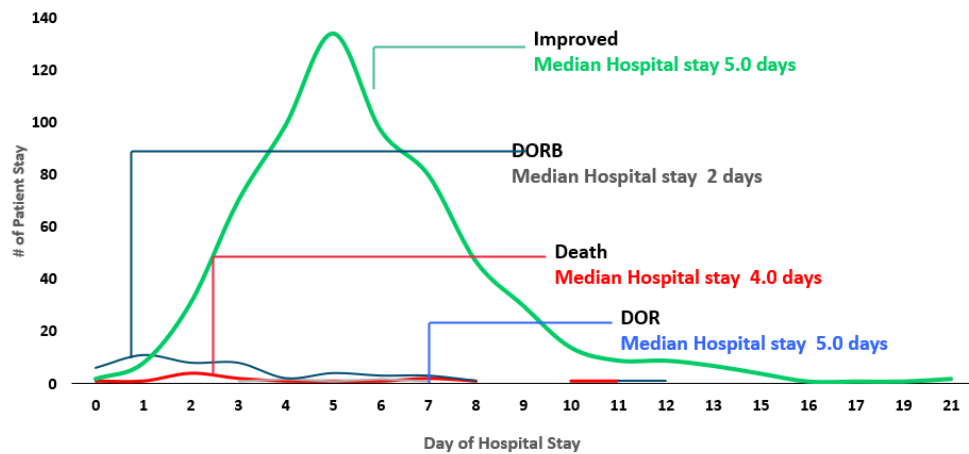
Figure 15 Distribution of disease outcome by age



4.4.8 Association between Hospital Stay and Patient's Outcome

Regarding the outcome of dengue infection, it was observed that the maximum number of patients recovered from the disease and the median hospital stay was 5 days, DORB (Discharge of risk bond) condition, of which the median hospital stay was 2 days, patients died with a median hospital stay of 4 days, and 1% were in DOR (Discharge of risk) conditions with a median hospital stay of 5 days (Figure 16).

Figure 16 Distribution of disease outcome by age



Section 4.5: Coinfection status of the children

Since all the patients manifested respiratory symptoms, we tested 131 nasopharyngeal swab samples for influenza and RSV from dengue suspected cases. Among the 131 cases, seven were co-infected with dengue and influenza A, four were co-infected with dengue and influenza B, and two were positive for influenza A, B, and RSV. No coinfection between dengue and RSV was found in the study. Moreover, all the co-infections were found between influenza and DENV-2 serotypes. (Table 5)

Table 5: Distribution of the children by coinfection

Coinfection			
Pathogens	Influenza A (n = 9)	Influenza B (n = 6)	RSV (n = 2)
Dengue virus (n = 125)	7	4	0

Section 4.6: Screening and serotyping of Dengue virus using RT-PCR

Among 154 selected cases (104 RDT positive, 50 RDT negative). Among 104, 102 confirmed positive by RT-PCR. Out of 50 negatives, 23 were positive by RT-PCR. Serotypes done for 110 out of 125 RT-PCR-positive cases; 87% were dengue serotype-2 (DENV-2), and 13% were positive for dengue serotype-3 (DENV-3). However, we did not find any DENV-1 or DENV-4 serotypes. (Table 6), (Figure 7,18,19)

Fig: 17 Circulating serotypes/genotypes of Dengue Virus (2013-2022)

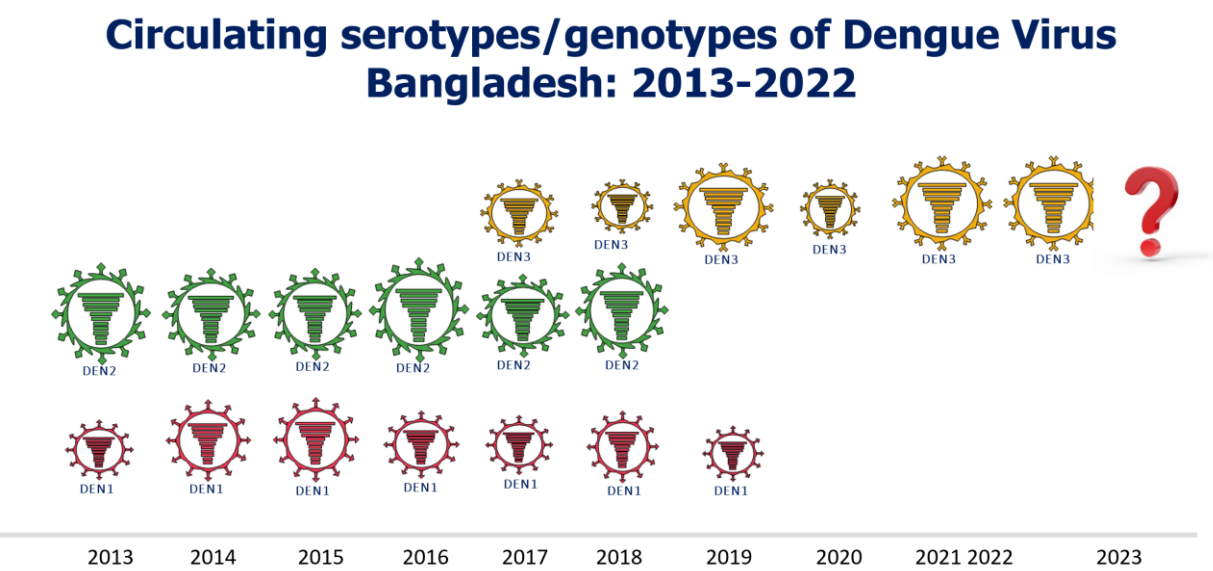


Fig: 18 Circulating serotypes/genotypes of Dengue Virus (2013-2022)

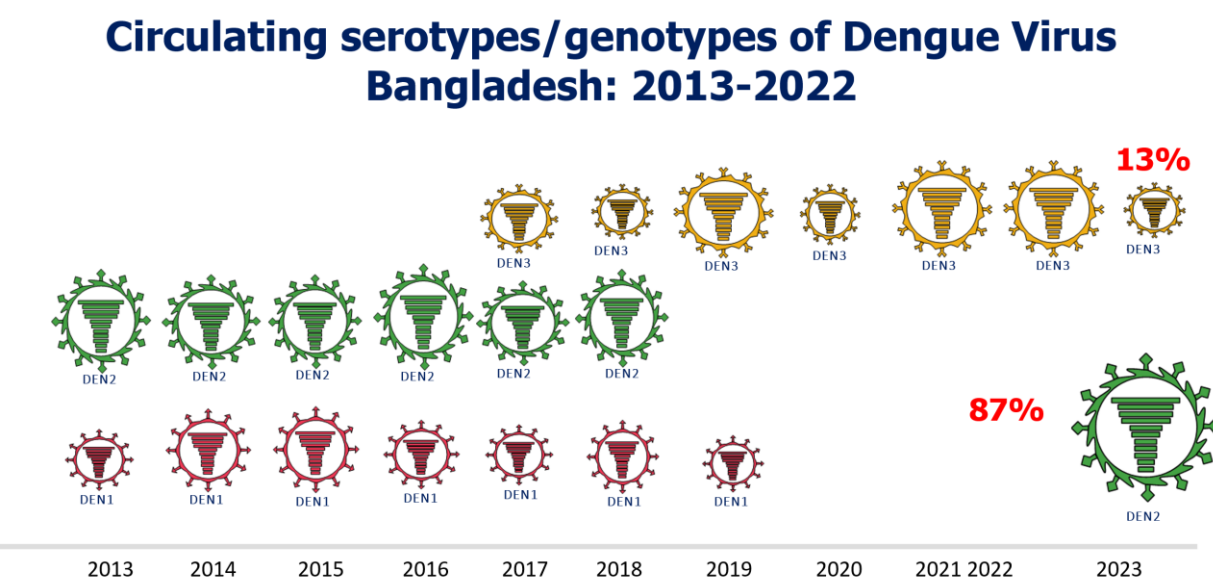


Fig: 19 Genomic Footprint of DEN2 and DEN3 Genotypes

Genomic Footprint of DEN2 and DEN3 Genotypes

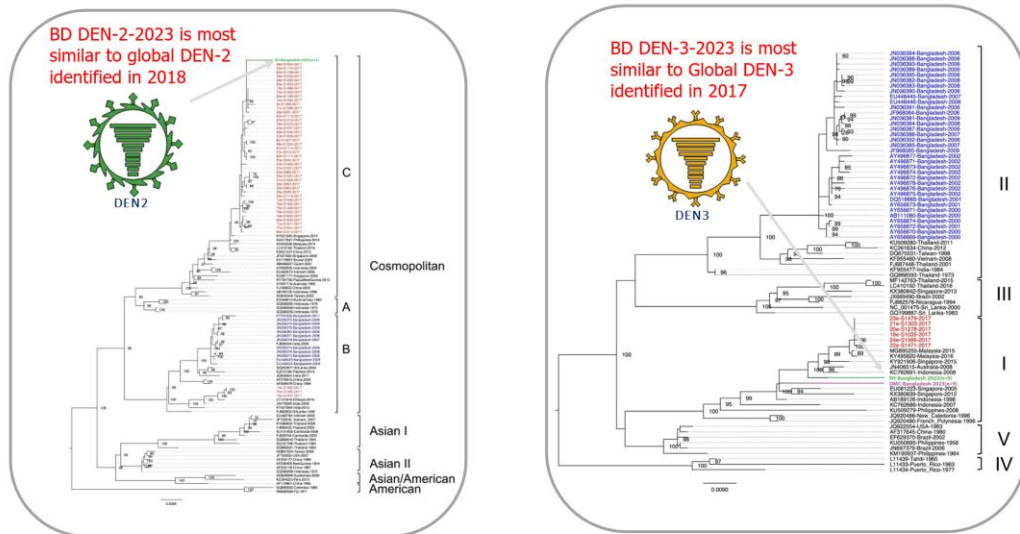


Table 6: Screening and serotyping of Dengue virus using RT-PCR

Screening		
Viral RNA	n	%
Positive	125	81
Negative	29	19
Serotyping		
Serotypes	n	%
DENV-2	98	87 %
DENV-3	12	13%

Section 4.7: Comparison of the diagnostic performance of rapid antigen tests to RT-PCR

Study findings show after comparing the diagnostic performance of rapid antigen test to RT-PCR, we found 34% false negative result. (Figure 17)

Figure 20 Diagnostic performance of rapid antigen tests to RT-PCR

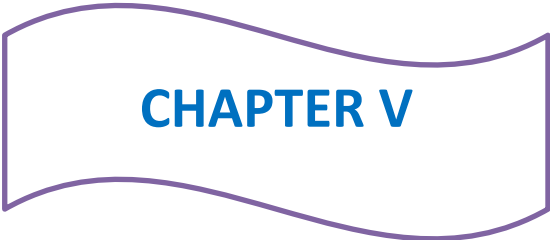
Dengue Rapid Tests vs Real-Time PCR



False Negative: 34%

		Rapid kit (NS1/IgM)		
		Positive	Negative	Total
Real-time PCR	Positive	96	17	113
	Negative	8	33	41
	Total	104	50	154

Rapid Test Status	False Positives	Rapid Test Status	False Negatives
NS1+ IgM Positive	2	NS1+ IgM Negative	12
NS1 Positive	4	NS1 Negative	2
IgM Positive	2	IgM Negative	3
Total	8	Total	17



CHAPTER V

DISCUSSION

CHAPTER-V

DISCUSSION

The cross-sectional study was conducted in Bangladesh Shishu Hospital, Dhaka among 722 participants aged 1-15 years for epidemiological, nutritional and genomic analysis; from July to October 2023 during the dengue epidemic's peak wave. NS1Ag and IgG, IgM for dengue test was used to diagnose dengue virus infected children. Our findings respective to study are described under the following headings.

Socio-demographic characteristics

The study revealed that 87% (n=600) of dengue infected patients were from Dhaka city, among them 82% (n=494) were from Dhaka North City Corporation. On the other hand, 13% patients were from outside Dhaka city. Among the dengue infected children 58% (n=422) were male and 42% female. Most affected age group was 1- <5 years (45%), whereas <1 year old children were in the least affected age group (13%) which is similar to another study conducted in Cameroon [Tchuandom SB et al., 2019]. The above findings indicate that infants and young children are very much susceptible to dengue.

Clinical Characteristics of Dengue

Our study revealed that all the participants presented with fever (100%), half of them had vomiting (49%), whereas abdominal pain (27%) and loose motion (19%) was also common. Other studies also presented similar findings [32,33,34,35]. The GI symptoms were also noted to be the most prevalent symptoms in children during outbreaks in 2006 and 2008 in Bangladesh (36). Furthermore, respiratory symptoms like cough, sore throat, and rhinorrhea have been observed in patients with dengue fever (24).

Investigating about the warning signs of dengue 71% children had the warning signs of dengue at presentation. Surprisingly 16% children with warning signs required ICU support whereas 11% required ICU who had no previous warning signs on admission. Studying the monthly

distribution of dengue cases highest number was recorded in August (380 cases, 53%) followed by July (291 cases 40%). Additionally, in tropical countries like Bangladesh, the period of the highest indexes of DENV cases (June-August) coincides with seasonal outbreaks of respiratory viruses. As a result, there is a high possibility of getting coinfecting with other viruses, like influenza, that have the same seasonality (April-July). In this respect, DENV circulation in endemic countries poses a diagnostic dilemma, especially during concurrent respiratory outbreaks.

Regarding associated comorbidity 10% (n=78) patients had comorbid conditions. Leading comorbid conditions were Obesity, Congenital Heart disease, Thalassemia, Vitamin D deficiency and Rickettsial fever respectively. Results from previous studies failed to show any solid consistency regarding the association between the nutritional status and dengue infection [Nguyen T et al., 2013]. In another study findings suggest that overweight children with dengue infection might be at a higher risk of developing a severe form of infection. Consequently, the effects of nutritional status (Malnutrition and Overweight/Obesity) on dengue disease outcome have been controversial (36). Shock was a major complication of dengue (17%) followed by Macrophage activation Syndrome (MAS, 3.2%), Pneumonia (2.6%) and Myocarditis. Among those with shock 96% children experienced compensated shock and 4% had decompensated shock.

Nutritional Characteristic

Among 722, Only 349 respondents up to age 5 years were enrolled. The study objective was to assess nutritional status by GMP card (wt for ht & wt for age) but height measurement was not performed due to respondent hemodynamic instability and unwillingness. However, this study revealed half of them found normal and 44% were in moderate underweight. Only 3% of them had severe underweight whereas 2% had overweight.

Impact of Hospital Stay

Study revealed that 71% patients stayed in hospital for 3 to 7 days. The maximum number of patients who recovered from dengue had a median hospital stay of 5 days; 2% patients who died had a median hospital stay of 4 days. Also, it is observed that earlier diagnosis was associated with less duration (5 days) of hospital stay. Early detection and timely treatment of

severe dengue can reduce dengue-associated mortality (28). This finding was also supported through a study conducted by (37). Less severe patients were likely to recover fast and were released from the hospital early (38).

82% (n=549) patients who stays in hospital received at least 1 antibiotic and 16% patients were treated with 2 different antibiotics. Among the administered antibiotics highest percentage was Levofloxacin (63%) followed by Meropenem (26%), Ceftriaxone (9%), Ciprofloxacin (7%) respectively. The length of hospital stay was positively correlated with the use of multiple antibiotics. Children with average stay of 5 days took only 1 antibiotic. According to the study findings >90% children (n=670) of all age group received antibiotics. This was a notable concern in the misuse of antibiotics in dengue management, and a study stated that antibiotic treatment did not result in a shorter hospital stay for Dengue patients (39). As dengue is a viral disease, antiviral treatment could be effective and reduce the use of antibiotics, as antibiotic resistance is a more common phenomenon at the current time. In this regard, physicians should be more concerned about the use of antibiotics in Dengue outbreaks.

Concerning the outcome of hospitalized dengue children 90% (n=648) recovered completely and 2%(n=17) children died. Highest percentage of death occurred in children under 1 year of age. For children under five years, deaths from other lower respiratory infections continue to be of more significant concern because their death rates are more than ten times higher than those from tuberculosis (25). Likewise, Asian nationwide surveillance data reveals that the most significant risk of severe dengue disease has always been associated with newborns under one-year-old and children between the ages of four and nine (40). Because of their undeveloped hemodynamic system, children, particularly newborns, are likely to acquire severe dengue disease (41,40). Dengue infections may progress to Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), which are associated with higher hospitalization and case fatality rates, particularly among children (1).

Coinfection Status

In our study we tested 131 children with respiratory symptoms for Influenza and RSV from dengue suspected cases. Among 131 cases, 7 patients were coinfectd with Influenza A, 4 with Influenza B. All coinfections were with DENV-2 serotype and Influenza. No coinfection between dengue and RSV was found in our study. A study found that a single diagnosis targeting DENV that is coinfectd with influenza A can increase the severity of diseases (5). In

tropical locations where influenza and dengue viruses commonly co-circulate, including Brazil, Thailand, and Vietnam, coinfection is a risk factor for severe disease (42,43,44,45,46).

Serotyping of Dengue Virus

In this cross-sectional study, 154 cases (104 RDT positive, 50 RDT negative). were selected for screening and serotyping by RT PCR. Among 104, 102 confirmed positive by RT-PCR. Out of 50 negatives, 23 were positive by RT-PCR. After comparing the diagnostic performance of rapid antigen test to RT-PCR, we found 34% false negative result.

Among 125 RT PCR positive cases serotyping was done for 110 cases. 87% cases were with dengue serotype-2 (DENV-2) and 13% cases were positive for dengue serotype-3 (DENV-3). However, we didn't find any DENV-1 or DENV-4 serotype. The pattern of DENV distribution is not unique. Several reports have documented that replication fitness, infection dynamics, and pathogenicity vary among DENV serotypes (7,8,9). For example, four dengue virus serotypes have been identified in Bangladesh (47), but the circulation pattern has changed over time. DENV-1 and DENV-2 were the dominating serotypes up to 2016 (10), followed by DENV-3, which was eventually responsible for the dengue outbreak in 2019 (11). Since the previous largest outbreak in 2019, DENV-3 has been the most prevalent circulating serotype in Bangladesh; however, DENV-2 mostly replaced it in 2023 (6). Co-circulation of several dengue serotypes increases the potential of secondary infection, which, because of the antibody-dependent enhancement (ADE) effect, may raise the risk of severe dengue (48,49,50,51,52).



CHAPTER VI

6.1 Conclusion

6.2 Recommendations

CHAPTER VI

6.1 Conclusion

The burden of dengue infection in children in Bangladesh is increasing, and the interplay between epidemiological, nutritional, and genomic factors complicates disease management and prevention. Epidemiological studies highlight the growing incidence of dengue in urban areas, while malnutrition exacerbates the severity of the disease. Findings revealed that more than 2/3rd children were from Dhaka north city corporation and male were more prone to dengue viral infection. All the children presented with H/O fever, and around half of them complained of vomiting. Notable symptoms among the children were abdominal pain, loose motion and cough of cases. More than 2/3rd of children had Dengue warning signs. But children having no warning signs also went to ICU. Children who had probable secondary infection need to hospital stay more. They had complications of shock, MAS, pneumonia, myocarditis, septicemia and pleural effusion. After genomic analysis and sequencing one-third of result found false negative. In case of RT-PCR dengue positive and negative cases had Flu or RSV infection. Most of the children affected by DEN-2. Additionally, genomic research suggests that genetic predispositions in children may influence their susceptibility to severe dengue manifestations. The study emphasizes the need for enhanced diagnostic accuracy, better management strategies, and targeted public health measures to address the high prevalence of pediatric dengue fever in Bangladesh, particularly among the most affected age groups. The findings of this study have important implications for public health because dengue fever and its associated death are currently a growing concern in Bangladesh.

6.2 Recommendations

The circulating serotypes and genotypes of dengue virus in Bangladesh exhibit significant variation, which is crucial for understanding the dynamics of dengue transmission and the factors that influence disease severity, particularly in children. The predominance of certain serotypes in different outbreaks and the genetic diversity of circulating strains underscore the complexity of managing dengue in Bangladesh. Based on the study findings following recommendation are founded:

- Enhanced surveillance systems that incorporate both serotype and genotypic analysis of circulating strains to track the evolution of the virus.
- Improved public health strategies to mitigate the risks posed by serotype co-circulation, such as better mosquito control and vaccination campaigns.
- Further research into the genetic markers associated with severe dengue outcomes, which could help in predicting and preventing severe disease in children.
- Feasibility of using Real-time PCR for dengue screening in the coming years and laboratory network system like COVID-19 should be established.
- During dengue seasons, other circulating respiratory viruses should also be tested because they develop similar symptoms.

By strengthening these areas, Bangladesh can improve its ability to manage and control dengue outbreaks, ultimately reducing the disease burden, especially in vulnerable populations such as children.



CHAPTER VII

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ANNEXURE- I

Seminar Report



Institute of Nutrition and Food Science
University of Dhaka
Dhaka-1000, Bangladesh

Phone : 9661920-73/8400, 8401
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Ref: INFS/2024-25/572

Date : 18/05/2025

May 18, 2025

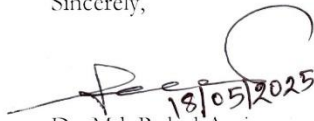
Re: Seminar Report of Kinkar Ghosh, MPhil Researcher

To whom it may concern,

This is to certify that MPhil student Kinkar Ghosh of the Institute of Nutrition and Food Science (INFS), University of Dhaka, bearing the registration no: 194, Session: 2020-2021; presented his MPhil work titled "Dengue Viral Infection in Children: Epidemiological, Nutritional, and Genomic Analysis" on 23-01-2025 at INFS auditorium.

Professor Dr. Khaleda Islam, Supervisor, along with other faculty members, were present during his seminar. Different opinions and suggestions were given by the faculty members. The researcher and his supervisor agreed to comply with the recommendations and suggestions, where possible, to incorporate finally in his thesis.

Sincerely,


18/05/2025

Dr. Md. Ruhul Amin

Professor

Member of the Seminar Committee of INFS

Institute of Nutrition and Food Science

University of Dhaka, Dhaka-1000, Bangladesh

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ruhul.infs@du.ac.bd


26.05.2025
Director
Institute of Nutrition & Food Science
University of Dhaka

ANNEXURE- II**Work Schedule**

Activities	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Protocol development and Institutional Review Board (IRB)/ERC approval												
Sample and data collection												
Sample testing												
Data analysis and reporting												
Report writing												

ANNEXURE- III

Ethical Clearance

BANGLADESH SHISHU HOSPITAL & INSTITUTE

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website : www.bshi.org.bd



বাংলাদেশ শিশু হাসপাতাল ও ইনস্টিটিউট

শেরেবাংলা নগর, ঢাকা-১২০৭, বাংলাদেশ

ফোন : (পিএবিএক্স) ৫৫০৫৯০৫১-৫৫০৫৯০৬০, ৫৫০৫৯০৬১, ৫৫০৫৯০৫০

ফ্যাক্স : ৮৮০-২-৫৫০৫৯০৬২, ই-মেইল : dhakashishu2010@gmail.com

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Notification

No. Admin/BSHI/2023/1775(1)

Date: 31/07/2023

To
Kinkar Ghosh
Epidemiologist
Bangladesh Shishu Hospital & Institute
Dhaka-1207

Subject: Ethical clearance for the research proposal entitled **“Epidemiology of Dengue Viral Infection in Pediatric Population in a Tertiary Care Pediatric Hospital in Bangladesh”**

Your research proposal protocol entitled **“Epidemiology of Dengue Viral Infection in Pediatric Population in a Tertiary Care Pediatric Hospital in Bangladesh”** has been reviewed and accepted by the Ethical Review Committee of Bangladesh Shishu (Children) Hospital & Institute. Your data collection period should be 6 months from the issuing date of ethical clearance letter.

Sincerely,

Prof. (Dr.) Reaz Mubarak
Member Secretary
Ethical Review Committee
&
Head, Department Epidemiology and Research
Bangladesh Shishu (Children) Hospital & Institute
Sher-E-Bangla Nagar, Dhaka-1207

Copy to: -

1. Director, BSHI
2. Deputy Director (Hospital), BSHI
3. Deputy Director (Finance), BSHI
4. Personal Secretary to Director, BSHI
5. Office copy

ANNEXURE- IV

Informed written consent in Bengali

JD: 12328398 / SL: 14 / Dipika / Sy. 6m
পিতামাতার সম্মতি পত্র

গবেষণা শিরোনাম : Dengue Viral Infection in Children: Epidemiological,
Nutritional and Genomic Analysis

সহযোগী সংস্থা: বাংলাদেশ শিশু হাসপাতাল ও ইনস্টিটিউট

গবেষণার উদ্দেশ্য : এই গবেষণার উদ্দেশ্য হল বাংলাদেশের টারশিয়ারী লেভেল হাসপাতালের শিশুদের মধ্যে ডেঙ্গু ভাইরাসের বিস্তার ও ব্যাপকতার সার্বিক চিত্র তুলে ধরা।

গবেষণার পটভূমি :

ডেঙ্গু একটি মশাবাহিত মারাত্মক সংক্রামক ব্যাধি। এডিস এজিন্টি এবং এডিস অ্যালবোপিকটাস মশার কামড়ে মানুষ বেশি সংক্রমিত হয়। ডেঙ্গু হল সবচেয়ে দ্রুত ছড়িয়ে পড়া মশাবাহিত রোগ যা জনস্বাস্থ্যের জন্য হুমকি হয়ে দাঁড়িয়েছে, বিশেষ করে বাংলাদেশ সহ গ্রীষ্মমণ্ডলীয় এবং উপ-ক্রান্তীয় দেশগুলির জন্য। বাংলাদেশ, দক্ষিণ এশিয়ার একটি জনবহুল দেশ যেখানে ১৬৭ মিলিয়নের বেশি লোক বসবাস করে। ২০০০ সালে প্রথমে বাংলাদেশে ডেঙ্গু সংক্রমণ ধরা পড়ে। তারপর থেকে এদেশে এ রোগের প্রাদুর্ভাব বেড়ে চলেছে। সঠিক প্রতিরোধমূলক কৌশলের অভাব, প্রাদুর্ভাব মোকাবেলায় অপর্যাপ্ত প্রযুক্তি এবং ডেঙ্গু সংক্রমণ সম্পর্কে সম্প্রদায়-স্তরের সচেতনতার অভাব জনস্বাস্থ্য বিপর্যয়ের কারণ। এসব ঝুঁকির কারণে, সাম্প্রতিক বছরগুলোতে বাংলাদেশ ধারাবাহিকভাবে বড় ধরনের ডেঙ্গু প্রাদুর্ভাবের সম্মুখীন হতে যাচ্ছে। এই গবেষণাটি বাংলাদেশ শিশু হাসপাতাল ও ইনস্টিটিউট অনুমোদন, অর্থায়ন ও নির্দেশনায় পরিচালিত হচ্ছে।

আপনাকে কেন এই গবেষণায় আমন্ত্রণ জানানো হয়েছে?

আপনাকে এই গবেষণায় আমন্ত্রণ জানানো হয়েছে কারণ আপনি বাংলাদেশের একজন দায়িত্বশীল নাগরিক ও একজন শিশুর অভিভাবক। কিছু গবেষণায় দেখা গেছে যে, ঘন জনসংখ্যা, অপর্যাপ্ত পয়ঃনিষ্কাশন এবং স্বাস্থ্যবিধি ব্যবস্থা বাংলাদেশের মতো দেশে ডেঙ্গুর প্রাদুর্ভাবকে দীর্ঘায়িত করছে। শিশুদের সংক্রমণের প্রবণতা বেশি হওয়া সত্ত্বেও দক্ষিণ এশিয়ায় বসবাসকারী শিশুদের ডেঙ্গু সংক্রমণের বিষয়ে প্রকাশিত তথ্যের পরিমাণ অপর্যাপ্ত। কারণ ছোট বাচ্চাদের বেশিরভাগ ডেঙ্গু সংক্রমণ উপসর্গবিহীন বা নূন্যতম লক্ষণীয়। বাংলাদেশী শিশুদের মধ্যে ডেঙ্গু ভাইরাসের বিস্তার ও ব্যাপকতার বর্তমান চিত্র নির্ধারণের জন্য বাংলাদেশের একজন দায়িত্বশীল নাগরিক হিসেবে আপনি আমাদেরকে আপনার সন্তানের স্বাস্থ্য বিষয়ক তথ্য প্রদানে অনুমতি দিতে পারেন। আপনার এই সহযোগিতা ভবিষ্যতে ডেঙ্গু রোগে আক্রান্ত শিশুদের রোগ সৃষ্টভাবে নির্ণয় ও চিকিৎসা প্রদানে সহযোগিতা করবে।

পদ্ধতি এবং কার্যপ্রণালী (অংশগ্রহণকারীর কাছ থেকে কি প্রত্যাশা?)

আপনি যদি রাজী থাকেন তাহলে নিচের বিষয়সমূহ অতর্জিত হবে

১. আমরা আপনার পরিবারের সামাজিক-জনসংখ্যা সংক্রান্ত তথ্য সংগ্রহ করব
 ২. আমরা আপনার কাছ থেকে আপনার শিশুর ডেঙ্গু রোগ সম্পর্কিত তথ্য সংগ্রহ করব
 ৩. তথ্য সংগ্রহকারীরা আপনার শিশু হতে রক্ত ও নাক ও গলার সংযোগস্থলের নিঃসৃত রসের নমুনা সংগ্রহ করবে
- আপনার এই তথ্যগুলো সংগ্রহ করতে আমাদের আনুমানিক ২০ মিনিটের মত সময় লাগবে। আপনার সুবিধা অনুযায়ী তথ্য সংগ্রহের সময় নির্ধারিত হবে।

সম্ভাব্য ঝুঁকি এবং সুবিধা

আমরা বিশ্বাস করি যে এই গবেষণাটি আপনার বা আপনার সন্তানদের জন্য কোনো শারীরিক, আর্থিক বা অন্য কোন প্রত্যক্ষ/পারোক্ষ ঝুঁকির কারন হবে না। আপনার কাছ থেকে সংগৃহীত তথ্য সর্বাধিক গোপনীয়তার সাথে সংরক্ষণ করা হবে। আমরা আপনার নাম, আপনার সন্তানের নাম বা আপনার পরিবারের সঠিক ঠিকানা কোথাও ব্যবহার করবো না। আমাদের তথ্য ব্যবহার করে আপনি পুনরায় সনাক্ত হবেন না।

এই গবেষণায় সম্ভাব্য গোপনীয়তা লঙ্ঘনের ঝুঁকি নূন্যতম। এই গবেষণায় অংশগ্রহণ করে আপনি সরাসরি উপকৃত হবেন না। তবে, স্বাস্থ্য ব্যবস্থাপনায় এই জাতীয় জরিপ বাংলাদেশে শৈশবকালীন অ্যাজমা প্রকোপের বর্তমান পরিস্থিতি সম্পর্কে গুরুত্বপূর্ণ তথ্য সরবরাহ করবে। পরিশেষে এই গবেষণায় আপনার অংশগ্রহণ আপনাকে, আপনার পরিবারকে এবং বাংলাদেশের জনগনকে উপকৃত করবে।

একান্ততা, নামহীনতা এবং গোপনীয়তা

আমরা যে তথ্য সংগ্রহ করছি তার গোপনীয়তা বজায় রাখব। আমরা শুধু মাত্র গবেষণার কাজে এই তথ্য ব্যবহার করবো এবং এই গবেষণার ফলাফল প্রকাশ করার সময় আপনার বা আপনার সন্তানের নাম কোথাও প্রকাশ করা হবে না। এই গবেষণার ফলাফল প্রকাশ করার সময় একান্ততা, নামহীনতা এবং গোপনীয়তা রক্ষা করে সর্বসাধারণ এবং গবেষণার অংশীদারদের সাথে প্রকাশ করা হবে।

তথ্যের ভবিষ্যৎ ব্যবহার

আপনার এবং আপনার সন্তানদের কাছ থেকে প্রাপ্ত তথ্য নামহীনভাবে শুধুমাত্র গবেষণার উদ্দেশ্যে ব্যবহার করা হবে।

অংশ না নেয়া এবং প্রত্যাখ্যান করার অধিকার

দয়া করে আমাদের স্বাচ্ছন্দ্য জানাবেন যে আমাদের গবেষক দলকে আপনার এবং আপনার সন্তানদের সাথে কথা বলা, সাক্ষাতকার নেওয়া এবং আপনার সন্তানের রক্ত ও ন্যাজোফ্যারেনজিয়াল সোয়াব এর নমুনা দিতে রাজি আছেন কিনা। এই গবেষণায় অংশগ্রহণ করা/না করা একান্ত আপনার ইচ্ছা।

ক্ষতিপূরণ বা প্রতিনিধানের নীতি

আমরা ধরে নিচ্ছি এই গবেষণায় অংশগ্রহণে কোন ঝুঁকি নেই, তাই এই গবেষণায় অংশগ্রহণের জন্য আপনাকে সরাসরি আর্থিক কোন সুবিধা প্রদান করা হবে না।

আপনার প্রশ্নের উত্তর/যোগাযোগ ব্যক্তি

গবেষণা সম্পর্কে আপনার প্রশ্ন থাকলে আমরা উত্তর দিব। এরপর ও এই গবেষণা সম্পর্কে আপনার আরও কিছু জানার থাকলে এই গবেষণার গবেষক এর সাথে যোগাযোগ করতে পারবেন। (যোগাযোগ- কিংকর ঘোষ, গবেষক, সংশ্লিষ্ট প্রকল্প, মোবাইলঃ ০১৭১১৩৪১২৩২)।

গবেষণা সম্পর্কে আপনার কোন অসন্তোষ থাকলে আপনি আইআরবি এর সদস্য এর সাথে যোগাযোগ করতে পারেন (মো: আসাদুজ্জামান, রেকর্ড অফিসার, মোবাইল: ০১৭৬২৩২৩২০৯)

আপনি যদি এই গবেষণা কার্যক্রমে অংশগ্রহণ করতে সম্মত হন তাহলে নীচে উল্লিখিত স্থানে আপনার স্বাক্ষর বা বাম হাতের বৃদ্ধাঙ্গুলির ছাপ দিন।

আপনার সহযোগিতার জন্য আপনাকে ধন্যবাদ।

নামক

অংশগ্রহণকারীর স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির ছাপ

তারিখ ২৪/০৮/২৩

স্বাক্ষর

স্বাক্ষরী স্বাক্ষর অথবা বাম হাতের বৃদ্ধাঙ্গুলির ছাপ

তারিখ ২৪.০৮.২৩

খালেদুর রহমান

খালেদুর

প্রধান গবেষক বা প্রতিনিধির স্বাক্ষর

তারিখ ২৪.০৮.২৩

(নোট: প্রধান গবেষকের প্রতিনিধির ক্ষেত্রে সে তার পুরো নাম, পদবিসহ এবং স্বাক্ষর করবেন)

ANNEXURE- V

Questionnaire in English

Survey Questionnaire

Study Title: Dengue Viral Infection in Children: Epidemiological,
Nutritional and Genomic Analysis

Collaborating Agency: Bangladesh Shishu Hospital & Institute

IPN ID

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Study ID

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SECTION A: IDENTIFYING INFORMATION	
II1. Interviewer's name and Id:	
II2. Day / Month / Year of interview:	___ / ___ / 2023
II3. Date of admission:	___ / ___ / 2023
II4. Date of discharge:	___ / ___ / 2023
II5. Hello, my name is (your name). We are from Epidemiology of Dengue Viral Infection in Pediatric Population in a Tertiary Level Hospital of Bangladesh. We are conducting a survey to observe epidemiological characteristics of Dengue Viral Infection in Pediatric Population in Bangladesh. I would like to talk to you about these subjects. This interview usually takes about 20 minutes. Following this, I may ask to conduct interviews with you. All the information we obtain will remain strictly confidential and anonymous. If you do not wish to answer a question or stop the interview, please let me know. May I start now? In the hard copy of questionnaire, please make a circle (o) around the correct option.	
II6. Patient's name:	II7. Age (years, months, days): _____
II8. Date of birth: ___ / ___ / ___	II9. Blood group:
II10. Respondent's name and mobile number: Name: _____ Mobile number: _____	
II11. Gender of the respondent: Male.....1 Female.....2 Hizra.....3	
II12. Address: (Upazilla, Zilla)	

II13. Area: Rural 1 Urban 2	II14. Division: Barisal 1 Chittagong 2 Dhaka 3 Khluna 4 Mymensingh 5 Rajshahi 6 Rangpur 7 Sylhet 8
II15. District:	
✓ II16. Where did the patient come from?	Inside Dhaka=1 Outside Dhaka=2 If the patient came from inside Dhaka, please answer the Q no. II17 OR If the patient came from outside Dhaka, please answer the Q no. II18
✓ II17. From which City corporation does the patient come?	North City corporation=1 South City corporation=2
II18. From which City corporation does the patient come? Please mention the name.	
SECTION B: HOSPITAL RELATED INFORMATION	
HI1. How you came to this hospital?	Self 1 Referral 0 If question 1 answer is referral then please go to Q no. HI2
HI2. From where you refer, mention the name?	
SECTION C: TRAVEL HISTORY (In case of outside Dhaka patient)	
HT1. Is there any travel history?	Yes 1 No 0
SECTION D: DEMOGRAPHIC CHARACTERISTICS	
✓ DC7. What is the religion of head of the household?	Muslim 1 Hindu 2 Christianity 3 Buddhism 4 Other religion (specify) 6 No religion 7

Dengue related information of the child			
SI No.	Question English	Value	Relevant
SECTION E: VARIABLES RELATED TO SYMPTOMS AND SIGNS			
01	Chief complaint/s		
02	Duration of fever (days)		
03	Does your child/infant have headache?	Yes=1 No=0 DK=2	

04	Does your child/infant have retro orbital pain?	Yes=1 No=0 DK=2	
05	Does your child/infant have body ache?	Yes=1 No=0 DK=2	
06	Does your child/infant have abdominal pain?	Yes=1 No=0 DK=2	
07	Does your child/infant have repeated vomiting/vomiting?	Yes=1 No=0 DK=2	
08	Does your child/infant have reluctant to feed?	Yes=1 No=0 DK=2	
09	Does your child/infant have a loss of appetite?	Yes=1 No=0 DK=2	
10	Does your child/infant have watery stool/loose stool?	Yes=1 No=0 DK=2	
11	Does your child/infant have runny nose?	Yes=1 No=0 DK=2	
12	Does your child/infant have cough?	Yes=1 No=0 DK=2	
13	Does your child/infant have difficulty in breathing?	Yes=1 No=0 DK=2	
14	Does your child/infant have rash?	Yes=1 No=0 DK=2	
15	Does your child/infant have bleeding from any place?	Yes=1 No=0 DK=2	
16	How would you describe your child/infant activity level since illness began?	Normal=1 Less active/ Irritable=2 Difficult to get out of bed=3	
17	Does your child/infant have stiff neck?	Yes=1 No=0 DK=2	
18	Does your child/infant have convulsion?	Yes=1 No=0	
SECTION D: VARIABLES RELATED TO DETERMINE THE RECOVERY PERIOD (Fill up by Investigator)			
19	How many days the child/infant need to recover?	Yes=1 No=0	
SECTION E: VARIABLES RELATED TO DENGUE COMPLICATIONS (Fill up by Investigator)			
20	Does the child/infant suffering from Dengue complications?	Yes=1 No=0	
21	If yes, mention the type.		

✓ D Diagnosis

SECTION F: VARIABLES RELATED TO ICU/PICU/RICU			
22	Does your child/infant admitted to ICU/PICU/RICU?	Yes=1 No=0	
SECTION G: VARIABLES RELATED TO HISTORY OF PAST ILLNESS			
23	Does your child/infant diagnosed with Dengue fever before this illness/ IgG positive?	Yes=1 No=0 DK=2	
SECTION H: VARIABLES RELATED TO HISTORY OF PAST MEDICATION			
24	Have your child/infant received any antibiotics within one week? <i>Antibiotic Received or not</i>	Yes=1 No=0 DK=2	
25	If yes, please specify the name <i>taken</i>		
SECTION I: VARIABLES RELATED TO OUTCOME			
26	What is the outcome of the child?	Recover=1 DORB=2 DOR=3 Death=4 Referred=5	

Thanks for your valuable time and information



Dengue Viral Infection in Children: Epidemiological Nutritional and Genomic Analysis



Specimen collection and transport form

1. Hospital ID:

Study ID:

Specimen Collection Date:

Name:

Gender: ☐ Male ☐ Female ☐ Transgender

Age: Year

Address: Mo:

2. Specimen Type	Collection Time	Lab Status	Code*
☐ Whole Blood (EDTA Tube)	:	☐ Accepted ☐ Rejected	
☐ Blood Serum (Plain Tube)	:	☐ Accepted ☐ Rejected	
☐ Nasopharyngeal Swab (VTM)	:	☐ Accepted ☐ Rejected	

*Specimen rejection code:

- 1: Unlabeled vial
- 2: Specimen ID dose not match
- 3: Blood is hemolyzed/ clotted
- 4: Leaking specimen
- 9: Other, specify:

3. Rapid Test Results

a. NS1	Positive Negative Invalid
b. IgG	Positive Negative Invalid
c. IgM	Positive Negative Invalid

4. Specimen Transport Time

H H M M

Specimen Receiving Date

Specimen Receiving Time

H H M M

Name

Signature

Porter:

Received by (Lab staff):