**Title: A Case-Control Study to Investigating the Association Between Comorbidities and the Development of Severe Dengue in Adult Patients in Dhaka, Bangladesh**

**Background of the study:**

**Dengue fever is an acute febrile viral disease transmitted by the bite of Aedes mosquitoes carrying any one of the four dengue viral serotypes. Approximately half of the world's population is at risk, especially in tropical and subtropical climates such as Bangladesh. The overall incidence of dengue has increased 30-fold over the past 50 years (2). About 390 million dengue infections are estimated to occur annually, of which a quarter of the cases (67–136 million) will manifest clinically (1). The first documented dengue epidemic occurred in the 1780s and was recorded almost simultaneously in Asian, African, and North American regions.**

**In Asia, a severe dengue outbreak was reported in the 1950s in the Philippines and Thailand, and in 1964 Bangladesh experienced a dengue outbreak for the first time, at which time the name Dacca fever was coined. Between 1964 and 1999, sporadic cases and small outbreaks clinically suggestive of dengue occurred across the country but were not officially reported, even though 5551 people were affected with 93 accounted fatalities (4). The first official outbreak of dengue fever in Bangladesh was in 2000, and since then the number of hospitalized patients has exceeded 3000 patients six times (4-6). In parallel with the major epidemics in 2018 and this year's outbreak, government surveillance systems have officially documented deaths with a clear predominance of cases and fatalities during the summer months (6,7). The geographical spread** with the largest having occurred **of the cases reported in 2019 affects all districts of the country, exhibits a clear predominance in men, and primarily encompasses younger adults**.

As of 20 November 2022, a total of 52807 laboratory-confirmed dengue cases and 230 related deaths have been reported by the Ministry of Health & Family Welfare of Bangladesh since 1 January 2022 with a case fatality rate (CFR) of 0.44%. The most affected division is Dhaka, accounting for 70.6% of cases and 60.4% of deaths. Dhaka city, the largest city in Bangladesh, located in the Dhaka division, has reported 64.5% (n= 34 071) of the total number of cases. Other affected divisions include the Chattogram division (13.2% of cases and 24.8% of deaths) and the Khulna division (5.5% of cases and 4.8% of deaths)

The high incidence of dengue cases this year is taking place in the context of an unusual amount of rainfall since June 2022, accompanied by high temperatures and high humidity, resulting in an increased mosquito population throughout Bangladesh. **As national surveillance is passive and only government hospitals are included, it is highly likely that substantial underreporting is taking place. Furthermore, the operational surveillance is not based on appropriate methods, such as the WHO projection done in July 2019.**

**We cannot underscore the seriousness of the current epidemic, which is, unfortunately, being handled with great laxity by the country's authorities, as shown not only by the marked under-reporting but also by the absence of health awareness campaigns targeting both the general public and health professionals. Such campaigns that aim for earlier and more consistent recognition and supportive clinical management of dengue cases are a major factor underlying reduced mortality for this highly contagious disease and are urgently needed (2). On the other hand,** Timely access to proper treatment for dengue patients by primary healthcare professionals not only reduces the number of unnecessary hospital admissions but also lowers fatality rates below 1%. The group progressing from non-severe to severe disease is difficult to define, but this is an important concern since appropriate treatment may prevent these patients from developing more severe clinical conditions. Very limited data is available in the literature about the association of demographic profile, clinical features, and comorbidities with the prognosis/complications of dengue in Bangladesh. The association will help in identifying high-risk dengue patients. Different warning signs can be used for early detection of potentially severe cases for timely treatment, to avoid unnecessary hospitalizations, and to decrease the case fatality of this disease. However, various clinical and epidemiological aspects have still not been completely elucidated, especially in Bangladesh. Henceforth, this study aims to understand the association of different clinical features and comorbidities. However, we only choose the Dhaka division because it is the largest city in Bangladesh and 70 % of dengue cases have occurred in Dhaka where 60% were death in the last year.

**Research question:**

1. What is the link between pre-existing medical conditions and the occurrence of severe dengue?
2. How are the clinical symptoms and laboratory test results upon admission related to the development of severe dengue?

**Methodology:**

**Hypothesis/objectives:**

1. The objective of this study is to examine the correlation between pre-existing medical conditions and the occurrence of severe dengue.
2. Additionally, we aim to explore the relationship between clinical symptoms and laboratory test results upon admission and the development of severe dengue.

**Case Definition:**

The diagnosis of dengue in this study was determined based on the 2009 World Health Organization (WHO) Dengue case classification, which includes laboratory-confirmed dengue or probable dengue. Laboratory-confirmed dengue cases will be identified through positive results for NS1 obtained using rapid dengue diagnostic kits. Probable dengue cases will be diagnosed based on clinical symptoms that met the WHO 2009 criteria for probable dengue, including fever along with at least two of the following: nausea or vomiting, rash, aches and pains, positive tourniquet test, leukopenia, and any warning signs. In addition to clinical criteria, probable dengue cases required either supportive serology results (IgM or IgG) or a positive Dengue IgM antibody in a serum sample taken during the late-acute or convalescent phase. Dengue serology results (IgM or IgG) will be obtained using dengue diagnostic kits. Severe dengue was defined according to the guidelines established by the WHO in 2009 **(Alexander N, Balmaseda A, Coelho ICB, Dimaano E, Hien TT, Hung NT, et al. Multicentre prospective study on dengue classification in four South-east Asian and three Latin American countries. Trop Med Int Health TM IH. 2011 Aug;16(8):936–48. pmid:21624014).**

**Study design and sample size:**

This case-control study will be conducted at Dhaka Medical College Hospital (DMCH), a prominent healthcare facility in Dhaka, Bangladesh. Specifically, a dedicated area on the ninth floor of DMCH has been allocated for the treatment and care of dengue patients. Participants will be collected between January 1st and June 30th, 2023, because the high incidence of dengue cases this year probably taking place in the context of an unusual amount of rainfall since June 2022. The sample size will be estimated as 435 participants using Sampsize, an online sample size calculator (https://sampsize.sourceforge.net/). For this case-control study for each group, we considered 80% of power, 95% level of confidence, 7% of exposure among controls, an Odds Ratio (OR) of 2.5, and a 1:2 allocation ratio. We determined that we would require 435 patients consisting of 145 cases and 290 controls. The percentage of exposed controls will be set at 7% as this is the prevalence of diabetes mellitus among the Bangladeshi population, which is also the comorbidity with the smallest prevalence among the comorbidities being studied) (http://bbs.portal.gov.bd/sites/default/files/files/bbs.portal.gov.bd/page/4c7eb0f0\_e780\_4686\_b546\_b4fa0a8889a5/HMSS.pdf). The odds ratio of 2.0 will be decided based on a previous study by Badawi et al who reported the odds ratio of severe dengue was around 2 to 4 for patients with comorbidities such as diabetes, hypertension, and heart disease (https://europepmc.org/article/med/29990356).

A case-control ratio of 1:2 would be chosen to enhance precision while considering feasibility. Controls will be selected from patients admitted to the general medical ward and matched the cases based on gender, age group (within a 5-year range of the case's age), and hospital admission date (within a 2-week timeframe of the patient's admission date). When multiple potential controls are available, up to five suitable controls will be considered. The top two controls will be then randomly selected using the randomization tool to minimize selection bias. The limit of five potential controls will be set due to practical considerations. Matching based on age and sex will be conducted to account for potential variations in outcomes across different age groups and genders. Matching according to hospital admission dates will also deem necessary to prevent confounding factors related to variations in circulating dengue serotypes.

**Inclusion and** **exclusion criteria:**

**Inclusion criteria**

1. Cases: This study includes adults (18 years and older) who will be diagnosed with severe dengue according to the 2009 Dengue Classification defined by the World Health Organization. To provide clarity, all severe dengue patients in our research will be treated in the intensive care unit at the designated study locations.

1. Controls: The control group comprises adults (18 years and older) who will be diagnosed with non-severe dengue and did not progress to severe dengue during their hospital stay. At our research site, these individuals with non-severe dengue will be treated in a dedicated general medical ward.

**Exclusion criteria**

We will exclude dengue patients who will be pregnant.

**Variables:**

Dependent variable:

Severe dengue (Yes or No)

Independent Variables:

All relevant clinical data will be collected retrospectively from the original medical case notes and hematology/biochemical laboratory records of the patients. The extracted information included demographic details (age, gender, ethnicity, nationality, height, weight), pre-existing medical conditions (obesity, diabetes mellitus, hypertension, hyperlipidemia, chronic kidney disease, chronic pulmonary disease (including asthma or chronic obstructive pulmonary disease), and stroke), presenting signs and symptoms (fever, abdominal pain, diarrhea, vomiting, lethargy, musculoskeletal (MSK) symptoms (such as myalgia, arthralgia, or bone pain), chills or rigors, upper respiratory tract infection (URTI) symptoms (such as a runny nose, sore throat, or cough), bleeding manifestations (such as gum bleeding, nosebleeds, hemoptysis, hematemesis, melaena, or vaginal bleeding), headache, and skin rash), and hematological/biochemical laboratory parameters (hemoglobin (Hb), hematocrit (Hct), white cell count (WCC), and platelet count, while the biochemical laboratory results included urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, creatine kinase (CK), and lactate dehydrogenase (LDH)) upon admission. Obesity was defined as a body mass index (BMI) equal to or exceeding 27.5 kg/m², based on admission data, following the WHO's Guidelines. Other comorbidities will be documented based on the formal diagnoses provided in the patient records.

**Statistical Analysis:**

All variables will be entered into Microsoft Excel and analyzed using the R software. A comparison will be made between cases and controls regarding demographics, comorbidities, presenting signs and symptoms, and admission laboratory parameters. Categorical variables will be presented as numbers and percentages, while continuous variables will be reported as the median and interquartile range (IQR) due to their non-normal distribution if they are confirmed by the Kolmogorov-Smirnov test, otherwise, mean and standard deviation (SD) will be applied.

Descriptive analyses involved comparing categorical variables using Pearson's chi-square (χ2) or Fisher's exact test. If our continuous variables will not follow a normal distribution, then, the Wilcoxon rank-sum test (Mann-Whitney U test) will be used for analysis.

In the inferential analysis, a matched case-control design will be employed, and conditional logistic regression will be used to account for the matching criteria (age, gender, admission date) in our study. Univariate conditional logistic regression will be utilized to calculate the conditional odds ratio (cOR), while multivariable conditional logistic regression will be used to determine the adjusted conditional odds ratio (AcOR), taking potential confounders into account. The confounding effect will be minimized by adjusting for potential confounders identified during the univariate analysis. Variables with statistically significant differences (p<0.05) between cases and controls in the descriptive analysis will be considered potential confounders.

All tests will be conducted at a significance level of 5%. The results will present in tables, showing the conditional odds ratio (cOR) or adjusted cOR (AcOR), p-value, and 95% confidence interval (CI). To check the predictive accuracy of the final model, the area under the curve (AUC) of the receiver operating characteristic curve was used. We also used the Hosmer and Lemeshow goodness-of-fit test to provide an overview of the overall fit of the final model.

**Results:**

Table 1:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Non-severe dengue  (n=290) | Severe dengue  (n=145) | Total  (n=435) | P-value |
| **Demographic Profile** | | | | |
| **Age** |  |  |  |  |
| <29 | 150 (58.37) | 60 (43.48) | 210 (53.16) | 0.016 |
| 30-59 | 93 (36.19) | 70 (50.72) | 163 (41.27) |  |
| >59 | 14 (5.45) | 8 (5.80) | 22 (5.57) |  |
| **Gender** |  |  |  |  |
| Male | 190 (65.52) | 75 (51.72) | 265 (60.92) | 0.005 |
| Female | 100 (34.48) | 70 (48.28) | 170 (39.08) |  |
| **Marital Status** |  |  |  |  |
| Married | 120 (41.81) | 85 (59.44) | 205 (47.67) | <0.001 |
| Unmarried | 167 (58.19) | 58 (40.56) | 225 (52.33) |  |
| **Highest Education** |  |  |  |  |
| Higher Secondary or above | 7 (2.46) | 7 (4.96) | 14 (3.29) | 0.316 |
| Secondary | 50 (17.61) | 27 (19.15) | 77 (18.12) |  |
| Primary | 107 (37.68) | 58 (41.13) | 165 (38.82) |  |
| No education | 120 (42.25) | 49 (34.75) | 169 (39.76) |  |
| **Monthly Income** |  |  |  |  |
| Low | 140 (48.95) | 78 (56.52) | 218 (51.42) | 0.143 |
| Medium | 81 (28.32) | 27 (19.57) | 108 (25.47) |  |
| High | 65 (22.73) | 33 (23.91) | 98 (23.11) |  |
| **Comorbidities** | | | | |
| **Hypertension** |  |  |  |  |
| Yes | 28 (9.66) | 19 (13.10) | 47 (10.80) | 0.275 |
| No | 262 (90.34) | 126 (86.90) | 388 (89.20) |  |
| **Diabetes** |  |  |  |  |
| Yes | 14 (4.83) | 11 (7.59) | 25 (5.75) | 0.244 |
| No | 276 (95.17) | 134 (92.41) | 410 (94.25) |  |
| **Heart Disease** |  |  |  |  |
| Yes | 7 (2.41) | 6 (4.14) | 13 (2.99) | 0.320 |
| No | 283 (97.59) | 139 (95.86) | 422 (97.01) |  |
| **Other comorbidities (CKD, COPD, Strokes, etc.)** |  |  |  |  |
| Yes | 23 (7.93) | 23 (15.86) | 46 (10.57) | 0.012 |
| No | 267 (92.07) | 122 (84.14) | 389 (89.43) |  |
| **Sign and Symptoms** | | | | |
| **Abdominal Pain** |  |  |  |  |
| Yes | 107 (36.90) | 84 (58.33) | 191 (44.01) | <0.001 |
| No | 183 (63.10) | 60 (41.67) | 243 (55.99) |  |
| **Diarrhea** |  |  |  |  |
| Yes | 61 (21.03) | 33 (22.76) | 94 (21.61) | 0.680 |
| No | 229 (78.97) | 112 (77.24) | 341 (78.39) |  |
| **Vomiting** |  |  |  |  |
| Yes | 22 (7.58) | 14 (9.66) | 36 (8.28) | 0.460 |
| No | 268 (92.41) | 131 (90.34) | 399 (91.72) |  |
| **Lethargy** |  |  |  |  |
| Yes | 21 (7.27) | 28 (19.44) | 49 (11.32) | <0.001 |
| No | 268 (92.73) | 116 (80.56) | 384 (88.68) |  |
| **Headache** |  |  |  |  |
| Yes | 225 (77.59) | 110 (76.39) | 335 (77.19) | 0.780 |
| No | 65 (22.41) | 34 (23.61) | 99 (22.81) |  |
| **Rash** |  |  |  |  |
| Yes | 203 (70.00) | 103 (71.53) | 306 (70.51) | 0.742 |
| No | 87 (30.00) | 41 (28.47) | 128 (29.49) |  |
| **URTI Symptoms** |  |  |  |  |
| Yes | 29 (10.00) | 27 (18.62) | 56 (12.87) | 0.011 |
| No | 261 (90.00) | 118 (81.62) | 379 (87.13) |  |
| **Hemorrhagic Symptoms** |  |  |  |  |
| Yes | 13 (4.50) | 7 (4.90) | 20 (4.63) | 0.854 |
| No | 276 (95.50) | 136 (95.10) | 412 (95.37) |  |
| **MSK Symptoms** |  |  |  |  |
| Yes | 207 (71.38) | 108 (75.00) | 315 (72.58) | 0.426 |
| No | 83 (28.62) | 36 (25.00) | 119 (27.42) |  |

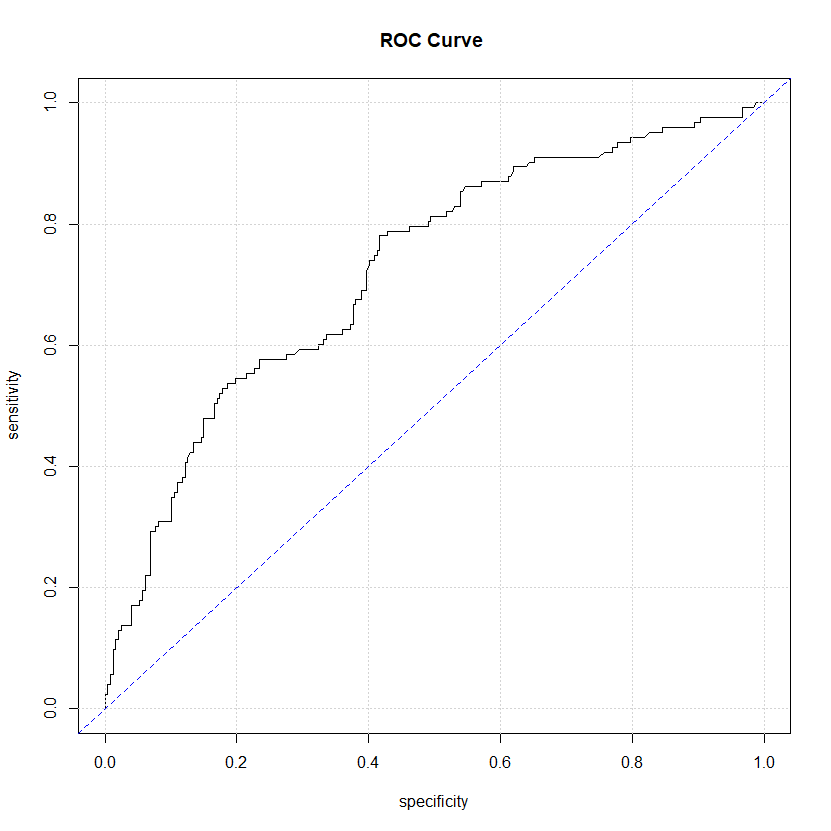
Table 2:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | COR (95% CI) | P-value | AOR (95% CI) | P-value |
| **Demographic Profile** | | | | |
| **Age** |  |  |  |  |
| <29 | 0.70 (0.28 – 1.83) | 0.447 | 1.29 (0.34 - 5.44) | 0.718 |
| 30-59 | 1.31 (0.53 – 3.46) | 0.558 | 1.34 (0.42 - 4.72) | 0.628 |
| >59 | Reference |  | Reference |  |
| **Gender** |  |  |  |  |
| Male | 0.56 (0.38 – 0.85) | 0.006 | 0.70 (0.42 - 1.17) | 0.171 |
| Female | Reference |  | Reference |  |
| **Marital Status** |  |  |  |  |
| Married | 2.04 (1.36 – 3.08) | <0.001 | 1.49 (0.70 - 3.14) | 0.296 |
| Unmarried | Reference |  | Reference |  |
| **Highest Education** |  |  |  |  |
| Higher Secondary or above | 2.45 (0.80 – 7.51) | 0.110 | 4.42 (0.84 - 27.64) | 0.086 |
| Secondary | 1.32 (0.74 – 2.34) | 0.340 | 1.92 (0.86 - 4.23) | 0.108 |
| Primary | 1.33 (0.84 – 2.11) | 0.228 | 1.56 (0.90 - 2.72) | 0.112 |
| No education | Reference |  | Reference |  |
| **Monthly Income** |  |  |  |  |
| Low | 1.10 (0.67 – 1.83) | 0.717 | 1.23 (0.66 - 2.32) | 0.523 |
| Medium | 0.66 (0.36 – 1.20) | 0.172 | 0.92 (0.46 - 1.87) | 0.824 |
| High | Reference |  | Reference |  |
| **Comorbidities** | | | | |
| **Hypertension** |  |  |  |  |
| Yes | 1.41 (0.75 – 2.61) | 0.276 | 0.89 (0.39 - 1.98) | 0.780 |
| No | Reference |  | Reference |  |
| **Diabetes** |  |  |  |  |
| Yes | 1.62 (0.70 – 3.65) | 0.248 | 0.79 (0.26 - 2.30) | 0.668 |
| No | Reference |  | Reference |  |
| **Heart Disease** |  |  |  |  |
| Yes | 1.75 (0.55 – 5.34) | 0.325 | 1.30 (0.34 - 4.93) | 0.695 |
| No | Reference |  | Reference |  |
| **Other comorbidities (CKD, COPD, Strokes, etc.)** |  |  |  |  |
| Yes | 2.19 (1.18 – 4.07) | 0.013 | 1.51 (0.71 - 3.18) | 0.279 |
| No | Reference |  |  |  |
| **Sign and Symptoms** | | | | |
| **Abdominal Pain** |  |  |  |  |
| Yes | 2.39 (1.60 – 3.61) | <0.001 | 2.24 (1.34 - 3.80) | 0.002 |
| No | Reference |  | Reference |  |
| **Diarrhea** |  |  |  |  |
| Yes | 1.11 (0.68 – 1.78) | 0.681 | 1.20 (0.66 - 2.18) | 0.543 |
| No | Reference |  | Reference |  |
| **Vomiting** |  |  |  |  |
| Yes | 1.30 (0.63 – 2.60) | 0.461 | 1.15 (0.48 - 2.67) | 0.743 |
| No | Reference |  | Reference |  |
| **Lethargy** |  |  |  |  |
| Yes | 3.08 (1.69 – 5.71) | <0.001 | 2.81 (1.36 - 5.88) | 0.006 |
| No | Reference |  | Reference |  |
| **Headache** |  |  |  |  |
| Yes | 0.93 (0.58 – 1.51) | 0.780 | 0.75 (0.42 - 1.36) | 0.338 |
| No | Reference |  | Reference |  |
| **Rash** |  |  |  |  |
| Yes | 1.08 (0.70 – 1.68) | 0.742 | 1.13 (0.66 - 1.95) | 0.665 |
| No | Reference |  | Reference |  |
| **URTI Symptoms** |  |  |  |  |
| Yes | 2.06 (1.16 – 3.64) | 0.013 | 2.27 (1.13 - 4.55) | 0.021 |
| No | Reference |  | Reference |  |
| **Hemorrhagic Symptoms** |  |  |  |  |
| Yes | 1.09 (0.40 – 2.73) | 0.853 | 0.93 (0.31 - 2.59) | 0.888 |
| No | Reference |  | Reference |  |
| **MSK Symptoms** |  |  |  |  |
| Yes | 1.20 (0.77 – 1.91) | 0.426 | 1.30 (0.76 - 2.26) | 0.345 |
| No | Reference |  | Reference |  |

The AUC value of 72.31% in Table 3 indicates that the classification accuracy is fairly acceptable. Furthermore, the model passed the Hosmer and Lemeshow goodness-of-fit test (value = 11.12, degrees of freedom = 8, P-value = 0.195), and did not find any lack of fit in the model.

Table 3Test for the goodness of fit and predictive accuracy of the final model

|  |  |  |
| --- | --- | --- |
| Hosmer and Lemeshow goodness of fit test | | |
| Value | Degrees of freedom | P-value |
| 11.12 | 8 | 0.195 |
| The area under the curve (AUC) of the receiver operating characteristic curve (ROC) | | |
| Value | 72.31% | |



**Ethical considerations**

Written permission will be taken from the concerned authority and also from the respondent before data collection. The investigator will explain to the respondents the purpose of the study before data collection.

**Expected outcomes**

Significant association with severe dengue cases and comorbidities between adult patients. We also hypothesize the association between pre-existing medical conditions, clinical symptoms, laboratory test results upon admission, and the development of severe dengue.

Reference:

1.Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013; 496: 504.

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