International Journal of General Medicine

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

NS1 Rapid Card Test for Dengue Detection: Insights from the 2023 Outbreak in Bangladesh

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Background: Dengue poses a serious public health challenge in Bangladesh, with the 2023 outbreak resulting in over 1700 deaths, the highest on record. Despite apparent symptoms, many patients tested negative on the NS1 rapid antigen test, the primary diagnostic tool. This study assesses the reliability of the NS1 rapid test results by analyzing its sensitivity, specificity, and predictive values using RT-PCR as the gold standard. It also explores how viral serotypes and secondary infections may impact test performance, especially in NS1-negative cases.

Methods: This cross-sectional study recruited febrile patients at two tertiary hospitals in Dhaka, Bangladesh, within 24 hours to 7 days of fever onset. Patients were selected based on physician-advised NS1 antigen testing, without restrictions on age, sex, or occupation. NS1 antigen test reports were collected from hospitals, and RNA testing via RT-PCR was conducted on all samples. Additional IgG and IgM tests were carried out for NS1-negative, RT-PCR-positive cases, and serotyping was performed on all RT-PCR -positive samples.

Results: Of the 194 samples tested, 65 (33.5%) were RT-PCR positive for dengue. Among these, 25 (38.5%) were NS1-negative. Most RT-PCR-positive cases were male (67.7%), with the 16–45 age group most affected. Symptoms like body aches (83.1%) and headaches (70.8%) were common. The predominant serotype was DEN-2 (97.5% in NS1-positive and 84% in NS1-negative cases), known for lower NS1 sensitivity. NS1 test sensitivity, specificity, PPV, and NPV were 61%, 97%, 91%, and 83%, respectively. Early IgG positivity in NS1-negative cases suggested secondary infections.

Conclusion: Despite the high specificity of NS1 rapid tests, moderate sensitivity demands alternative diagnostics like RT-PCR, which are crucial for better dengue management, especially in the presence of DEN-2 infections and associated secondary infections in Bangladesh.

Keywords: dengue, NS1 rapid test, RT-PCR, serotypes, DEN-2 infections

Introduction

Dengue is a mosquito-borne febrile illness that poses a major health threat in tropical and subtropical regions.[1,2] According to the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), dengue is endemic in over 100 countries, with 400 million infections, 100 million cases, and 21,000 deaths annually. Over the past 50 years, its incidence has risen 30-fold, spreading to new countries and expanding from urban to rural areas.[1–3] Dengue Virus (DENV), a Flavivirus with four distinct serotypes (DENV-1 to DENV-4), was first identified in 1964, but life-threatening dengue hemorrhagic fever (DHF) was reported in 2000. Since then, annual dengue outbreaks have become increasingly severe and have emerged as

Received: 30 December 2024
Accepted: 5 April 2025
Published: 9 April 2025

a significant public health concern globally as well as in Bangladesh. DHF, along with Dengue Shock Syndrome (DSS), can lead to plasma leakage, haemorrhage, and organ impairment, making early diagnosis and management crucial.[2] In Bangladesh, two of the most severe outbreaks occurred in 2019 and 2022. In 2019, there were 101,354 laboratory-confirmed dengue cases and 164 deaths. In comparison, while the total number of cases in 2022 was significantly lower at 61,089, the death toll rose to 269, indicating a sharp increase in dengue-related fatalities.[3,4] The situation worsened further in 2023 when Bangladesh faced its most devastating dengue outbreak in two decades, resulting in 1705 deaths, making it the deadliest dengue epidemic globally that year. From January 1 to August 7, 2023, the Ministry of Health and Family Welfare of Bangladesh reported 69,483 laboratory-confirmed cases and 327 deaths. By September 17, 2023, the death toll had surged to 822 and continued to climb.[3,5,6] A key factor contributing to these fatalities has been delays in diagnosis and treatment.

Traditionally, dengue diagnosis relies on detecting the dengue virus nonstructural protein 1 (NS1) rapid test within the first seven days of fever onset.[7] However, during the 2023 outbreak, an alarming trend emerged—many patients tested negative for the NS1 rapid test despite showing classic symptoms of dengue fever. In this context, an increasing number of individuals are opting for self-testing. Unfortunately, a negative NS1 result often leads them to mistakenly assume they are not infected with dengue, delaying proper medical intervention.[3–5] Several reasons account for negative NS1 test results. Timing is critical, as the NS1 antigen is typically detectable only within 5–7 days after fever onset, and its sensitivity drops significantly beyond this period.[5] The Dengue NS1 rapid test has lower sensitivity (50–80%) and specificity compared to enzyme linked immunosorbent assay (ELISA) (80–90%) and reverse transcriptase polymerase chain reaction (RT-PCR) (>95%), making it less reliable for diagnosis. While useful for quick screening, ELISA and RT-PCR remain superior for accurate dengue detection.[8,9] Additionally, the dengue virus serotype influences the accuracy of NS1 testing, with DEN-2 frequently producing lower sensitivity results, even within the early diagnostic window.[10–13] Moreover, in cases of secondary dengue infections, the sensitivity of NS1 testing tends to decrease due to interactions between the antigen and pre-existing antibodies.[14,15] To address these diagnostic challenges in the 2023 outbreak, the Society for Medical Virologists, Bangladesh, initiated this study with the aim of assessing the reliability of the NS1 rapid test by evaluating its sensitivity, specificity, and predictive values during the acute phase of infection. Insofar as Bangladesh, like any developing country, lacks funding, we compared the regular NS1 ICT test with the gold standard RT-PCR. We focused on RT-PCR-positive cases that tested negative for NS1 to assess the differences between the tests and the reliability of NS1 test reports.

By providing critical insights into these aspects, the study seeks to enhance diagnostic accuracy and guide effective dengue outbreak management strategies in Bangladesh. This comprehensive approach aims to strengthen early diagnostic practices, inform public health interventions, and ultimately reduce dengue-related mortality in future outbreaks.

Materials and Methods

This cross-sectional study was conducted between July and December 2023 for six (06) months. The patients were attended in the outpatient department (OPD) during August and September 2023. The febrile patients of all ages were recruited using a convenient sampling method based on specific inclusion and exclusion criteria. The inclusion criteria required blood collection within 24 hours to 7 days of fever onset, physician-advised NS1 antigen testing, and included individuals of any age, sex, and occupation. Patients with fever lasting more than 7 days or those unwilling to participate were excluded. Samples were collected from two tertiary care hospitals: Ahsania Mission Cancer and General Hospital, Ashulia, Dhaka, Bangladesh, and US Bangla Medical College Hospital, Narayanganj, Bangladesh. Patients from various regions across Bangladesh were referred to these hospitals. US Bangla Medical College Hospital is located in the Narayanganj District, which primarily serves the greater Narayanganj area, which is a very crowded business area of Bangladesh. This selection of hospitals was intended to ensure a diverse representation of the Bangladeshi population. The study was approved by the Ethical Committee of Ahsania Mission Cancer and General Hospital, Dhaka, Bangladesh, and conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants, granting permission to use leftover specimens for research purposes. For patients under 18 years of age, a parent or legal guardian provided informed consent.

Study Procedure

Demographic variables like age, gender, occupation and clinical information, including details on the patient's symptoms and medical history like fever, pattern of fever, headache, myalgia, retro-orbital pain, vomiting, nausea, abdominal pain and other clinical features were recorded at the time of blood sample collection. 5 mL of venous blood was collected from each patient with all aseptic conditions. Around 2 mL were taken in a tube for Immunochromatographic (ICT) and sent to the respective hospital lab, and 3 mL of blood was collected in an EDTA tube, gently mixed well and stored at −20°C for RT-PCR, serotyping and antibody analysis. Consultants or specialists in medicine advised NS1 tests and a doctor who completed the questionnaire was an MBBS graduate, part of a team led by a virologist.

NS1 Rapid Card Testing

NS1 antigen testing was performed on all patients using the available ICT kits in the hospital laboratories (Abbot: USA, Accurate: China). The primary aim was to evaluate the reliability of these routine laboratory reports by comparing the results with those obtained from RT-PCR testing, as this remains the gold standard in dengue diagnosis along with virus isolation and identification.[16] We collected the NS1 antigen test reports from the hospital laboratory for the study to assess their diagnostic accuracy.

RNA Extraction

RNA was extracted from plasma using the PureLink® Viral RNA/DNA Mini Kit. Whole blood, preserved in EDTA tubes at −20°C, was thawed and centrifuged at 1500–2500 rpm for 10–15 minutes to separate plasma. The plasma was carefully transferred and stored at −20°C or used immediately. For extraction, a lysis mixture (200 µL Lysis Buffer, 200 µL serum, 25 µL Proteinase K) was incubated at 56°C for 15 minutes, followed by ethanol addition and room temperature incubation. The lysate was passed through a spin column, washed, and RNA was eluted with 50 µL RNase-free water, then stored at −20°C for further use.

Qualitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Dengue RT-PCR was performed using the AltoStar® Dengue RT-PCR Kit (Germany). Reagents were thawed, vortexed, and centrifuged. A 31 µL master mix was prepared for each sample, including 5 µL Master A, 15 µL Master B, 1 µL Internal Control (IC), and 10 µL of the sample. Negative and positive controls (NTC and PC) were also processed. The QuantStudio™ 5 (USA) Real-Time PCR machine was used with the following program: reverse transcription (55°C, 20 min), denaturation (95°C, 2 min), and 45 cycles of amplification (95°C for 15 sec, 55°C for 45 sec, 72°C for 15 sec). Dengue DNA detection was based on FAM™ (dengue-specific) and VIC™/JOE™ (internal control) fluorescence channels. If both channels were negative, the test was repeated.

Dengue Serotyping

Dengue-positive patients were further tested for serotyping using the VIASURE Dengue Serotyping Real-Time PCR Detection Kit (Certest, Spain). The kit includes reagents for two multiplex reactions: one for DEN-1 and DEN-4 and another for DEN-2 and DEN-3. The PCR protocol involved reverse transcription at 45°C for 15 minutes, followed by denaturation at 95°C for 2 minutes, and 45 cycles of amplification at 95°C for 10 seconds and 60°C for 50 seconds (QuantStudio™ 5, USA). Results were interpreted based on detecting fluorescence in the specific channels for each serotype. Positive results for particular serotypes were confirmed through comparison with the positive and negative controls.

Dengue IgM and IgG Rapid Test

Only RT-PCR-positive but NS1-negative patients were tested for dengue IgM and IgG antibodies using the Bioline Dengue IgM/IgG ICT Kit. A 10 µL plasma sample was applied to the specimen well of the device, followed by 4 drops of diluent in the specimen well. The test was incubated for 15 to 20 minutes, after which results were interpreted by comparing the test line with the control line.

Statistical Analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population, including gender, age, and symptom distribution. The Chi-Square test was applied to compare the prevalence of symptoms between Dengue positive/negative, NS1-positive and NS1-negative groups, identifying significant differences in symptoms such as body aches and headaches. The diagnostic performance of the NS1 test was assessed using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Sensitivity and specificity were calculated to evaluate the accuracy of the test in identifying dengue-positive and dengue-negative cases, respectively. PPV and NPV were calculated to determine the likelihood of true positive and true negative results based on the test outcomes.

Results

This study analyzed 194 fever patients with dengue-like symptoms, with 65 (33.5%) cases confirmed as dengue-positive by RT-PCR, while only 44 (22.68%) cases were NS1 positive. Of the NS1-positive cases, 40 cases were also RT-PCR positive, and 4 cases were RT-PCR negative. Notably, 25 (38.46%) of the RT-PCR-positive cases were NS1-negative (Table S1). In dengue RT-PCR positive cases, males remained more than females (67.7% and 32.5%). In RT-PCR negative cases, those up to 30 years suffered more, but those 16 to 45 years of age remained the most sufferers in dengue RT-PCR positive cases. Major sign symptoms in dengue RT-PCR positive cases were body aches (83.07%), headache (70.77%), vomiting (55.38%), and cough (55.38%), which was not much different than dengue RT-PCR negative cases. Most dengue RT-PCR positive and negative patients came within 5 days of onset of fever (93.85% and 94.57%) (Table 1). The NS1 test demonstrated an overall sensitivity of 61.0%, specificity of 97%, PPV of 91%, and NPV of 83.0% when performed within 7 days of fever onset, highlighting its high reliability in confirming dengue in positive cases but limited effectiveness in ruling out the disease in negative cases. Testing within the first 5 days of fever showed slightly improved sensitivity (65.57%) and comparable specificity (96.75%), emphasizing that the diagnostic performance remains consistent and only marginally better when conducted earlier within 5 days, though no statistical significance was found.

Among dengue RT-PCR-positive patients, 25 (38.46%) patients were NS1-negative. Dengue RT-PCR and NS1 positive had a significantly higher (77.5%) proportion of males than NS1 negative cases (52%), where the gender distribution was more balanced. In RT-PCR positive, NS1 positive cases, the majority (82.5%) fell within the 16 to 45 age range, while RT-PCR positive but NS1 negative cases had a higher proportion of younger patients (0 to 15 years at 36.0%). RT-PCR positive, NS1-positive patients showed a higher prevalence of headache (92.5%), body aches (85.0%), and vomiting (60.0%) compared to NS1-negative patients. RT-PCR positive NS1-negative patients had more cases of runny nose (40.0%) and loose motion (16%). Cough was similarly reported in both groups, and no cases of rash or bleeding were found. All RT-PCR positive, NS1-positive patients reported fever lasting up to 5 days, whereas a small proportion (16.0%) of NS1-negative patients had reported with fever for more than 5 days. DEN-2 was the dominant

Table 1 Demographic Characteristics Among Dengue RT-PCR Positive and Negative Patients (n=194)

Variables		Dengue RT-PCR		OR (95% CI)	P value
		Negative	Positive		
Gender	Male	67 (52.0%)	44 (67.7%)	Reference	
	Female	62 (48.0%)	21 (32.3%)	2.2 (1.18 to 4.15)	0.012
Age Group	0 to 15 Years	51 (39.5%)	11 (16.9%)	Reference	
	16 to 30 Years	34 (26.3%)	23 (35.3%)	8.0 (2.55 to 25.18)	<0.001
	31 to 45 Years	22 (17.1%)	23 (35.3%)	5.6 (1.67 to 18.87)	0.005
	> 45 Years	22 (17.1%)	8 (12.3%)	2.9 (0.72 to 11.712)	0.13

(*Continued*)

Table 1 (Continued).

Variables		Dengue RT-PCR		OR (95% CI)	P value
		Negative	Positive		
Symptoms	Headache	56 (43.4%)	46 (70.7%)	3.2 (1.68 to 6.01)	<0.001
	Body ache	97 (75.2%)	54 (83.0%)	1.7 (0.78 to 3.57)	0.17
	Vomiting	53 (41.1%)	36 (55.3%)	1.5 (0.83 to 2.72)	0.18
	Loose Motion	6 (4.6%)	7 (10.7%)	2.4 (0.78 to 7.50)	0.12
	Runny Nose	57 (44.1%)	23 (35.3%)	0.8 (0.41 to 1.39)	0.37
	Sore Throat	33 (25.5%)	10 (15.3%)	0.6 (0.27 to 1.23)	0.14
	Cough	82 (63.5%)	36 (55.3%)	0.7 (0.39 to 1.31)	0.27
	Rash	3 (2.3%)	0 (0.0%)	–	–
	Bleeding	0 (0.0%)	0 (0.0%)	–	–
Duration of fever prior blood collection	≤ 5 Days	122 (94.6%)	61 (93.9%)	Reference	
	> 5 Days	7 (5.4%)	4 (6.1%)	1.1 (0.31 to 3.95)	0.86

Notes: P value reached from the Chi-square test, and P ≤ 0.05 is considered significant.
Abbreviations: RT-PCR, Reverse Transcription Polymerase Chain Reaction; n, sample size; OR, Odds Ratio; CI, Confidence Interval.

serotype, found in 97.5% of RT-PCR positive, NS1-positive cases and 84.0% of RT-PCR positive but NS1-negative cases. DEN-3 was present in 16% of NS1-negative but RT-PCR positive cases. A single case was co-infected with DEN-2 and DEN-3 (Table 2).

Day-wise, IgM and IgG positivity of NS1 negative, but RT-PCR positive cases showed IgM antibodies begin to appear around day 5 of fever onset, with their detection rate increasing significantly by days 6 and 7. Conversely, IgG antibodies are occasionally detectable as early as day 2 in some cases, likely due to secondary infections, but their presence does not show a consistent pattern beyond the early days. These results suggest that IgM is more reliable for diagnosing primary infections after day 5, while early IgG positivity can serve as a marker for secondary infections in NS1-negative patients (Table 3).

Table 2 Demographic Characteristics of Dengue RT-PCR Positive Patients, Categorized by NS1 Positive and NS1 Negative Cases (n=65)

Variables		Dengue RT-PCR Positive		OR (95% CI)	P value
		NS1 Positive	NS1 Negative		
Gender	Male	31 (77.5%)	13 (52.0%)	Reference	
	Female	9 (22.5%)	12 (48.0%)	2.8 (1.38 to 6.04)	0.005
Age Group	0 to 15 Years	2 (5.0%)	9 (36.0%)	–	0.001
	16 to 30 Years	19 (47.5%)	4 (16.0%)	–	
	31 to 45 Years	14 (35.0%)	9 (36.0%)	–	
	>45 Years	5 (12.5%)	3 (12.0%)	–	

(*Continued*)

Table 2 (Continued).

Variables		Dengue RT-PCR Positive		OR (95% CI)	P value
		NS1 Positive	NS1 Negative		
Clinical Symptoms	Headache	37 (92.5%)	9 (36.0%)	0.1 (0.04 to 0.26)	<0.001
	Body ache	34 (85%)	19 (76.0%)	0.7 (0.31 to 1.72)	0.469
	Vomiting	24 (60%)	12 (48.0%)	0.5 (0.29 to 1.14)	0.115
	Lose Motion	3 (7.5%)	4 (16.0%)	0.6 (0.18 to 2.18)	0.471
	Runny Nose	13 (32.5%)	10 (40.0%)	1.3 (0.69 to 2.75)	0.367
	Sore Throat	8 (20%)	2 (8.0%)	1.1 (0.51 to 2.69)	0.688
	Cough	22 (55%)	14 (56.0%)	1.4 (0.72 to 2.84)	0.293
	Rash	0 (0.0%)	0 (0.0%)		
	Bleeding	0 (0.0%)	0 (0.0%)	0.8 (0.71 to 0.83)	0.344
Days of fever prior blood collection	≤ 5 Days	40 (100.0%)	21 (84.0%)	Reference	
	> 5 Days	0 (0.0%)	4 (16.0%)	0.8 (0.70 to 0.82)	0.064
Serotyping	DENV 2 (60)	39 (97.5%)	21 (84.0%)	0.2 (0.02 to 2.14)	0.153
	DENV 3 (4)	0 (0.0%)	4 (16.0%)	–	–
	DENV 2 and 3 (1)	1 (2.5%)	0 (0.0%)	–	–

Notes: P value reached from the Chi-square test, and $p \leq 0.05$ is considered significant.
Abbreviations: RT-PCR, Reverse Transcription Polymerase Chain Reaction; NS1, Non-structural Protein 1; DENV, Dengue Virus; n, sample size; OR, Odds Ratio; CI, Confidence Interval.

Table 3 Day Wise Positivity of IgM and IgG in Cases That Are NS1 Negative but RT-PCR Positive for Dengue (n=25)

Days since Onset of Fever / Number of NS1 Negative But Dengue RT-PCR Positive Samples	IgM N=25		IgG N=25	
	Positive	Negative	Positive	Negative
Day 1 / 0 sample	00	00	00	00
Day 2 / 6 samples	0 (0.0%)	6 (100%)	1 (16.67%)	5 (83.3%)
Day 3 / 5 samples	0 (0.0%)	5 (100%)	1 (20%)	4 (80%)
Day 4 / 5 samples	0 (0.0%)	5 (100%)	1 (20%)	4 (80%)
Day 5 / 5 samples	1 (20%)	4 (80%)	1 (20%)	4 (80%)
Day 6 / 3 samples	2 (66.7%)	1 (33.3%)	0 (0.0%)	3 (100%)
Day 7 / 1 sample	1 (100%)	0 (0.0%)	0 (0.0%)	1 (100%)

Abbreviations: IgM, Immunoglobulin M; IgG, Immunoglobulin G; RT-PCR, Reverse Transcription Polymerase Chain Reaction; NS1, Non-structural Protein 1; n=25.

Discussion

This study provides essential insights into the diagnostic performance and limitations of the NS1 rapid card test, particularly in the context of the ongoing Dengue outbreak. In this study, the NS1 rapid test, advised by physicians and conducted in hospital laboratories to detect dengue NS1 protein in blood as an indicator of dengue infection,[7] demonstrated a sensitivity of 61.0% and a specificity of 97.0%, using RT-PCR as the gold standard. Performed within the

first seven days of fever onset, the NS1 rapid test also exhibited a PPV of 91.0% and an NPV of 83.0%, highlighting its high reliability in confirming dengue in NS1-positive cases. However, its moderate sensitivity limits its ability to rule out the disease in NS1-negative cases.

A recent study in Bangladesh reported a sensitivity of 68.3% and specificity of 100.0%, further emphasising the diagnostic reliability of NS1 positivity but reiterating the challenges in sensitivity.[17] These observations align with earlier studies, which also noted the limited sensitivity of NS1 testing.[18,19] Interestingly, testing within the first 5 days of fever onset showed a slight improvement in sensitivity (65.57%) with a comparable specificity of 96.75%. This trend supports the hypothesis that the NS1 antigen is most detectable during the acute phase of infection, as documented in previous research, including Hu and coworker's study findings.[20–22] We found high specificity across all time frames reinforces that NS1 positivity strongly indicates an actual Dengue infection; however, its moderate sensitivity highlights the need for additional tests, like RT-PCR or serological testing (IgM/IgG), especially for NS1-negative cases.

Dengue RT-PCR positive patients in this study were predominantly male (67.69%), compared to Dengue RT-PCR negative cases, where gender distribution was more balanced. This study found that most RT-PCR positive, NS1-positive patients were aged 16–45 years, while RT-PCR positive but NS1-negative cases were more common among children (0–15 years), suggesting that immune maturity and prior exposure influence NS1 antigenemia.[23] Although NS1 sensitivity is generally higher in primary infections, some children may experience early immune clearance of NS1, especially in secondary infections, leading to more false-negative results.[24,25] Additionally, severe dengue, which is more frequent in children, may further reduce NS1 detectability due to the formation of immune complexes.[26] These findings emphasise the importance of alternative diagnostic tests in NS1-negative pediatric cases to prevent missed dengue diagnoses.

The symptom profile in dengue RT-PCR positive cases featured body aches (83.07%), headache (70.77%), and vomiting (55.38%) as the most common symptoms, consistent with established clinical features of Dengue fever.[27,28] Dengue RT-PCR positive and NS1-positive patients reported higher incidences of headache (92.5%) and body ache (85%) compared to dengue RT-PCR positive but NS1-negative patients, rather exhibited a higher prevalence of runny nose (40%) and loose motion (16%) though they were not clinically significant. These variations in clinical presentation suggest that symptom-based differentiation between NS1-positive and NS1-negative cases may not assist in guiding diagnostic decisions.

Bangladesh has experienced an alarming surge in dengue cases in recent years, with significant outbreaks in 2019 and 2022. However, 2023 emerged as the deadliest year on record, with the first half alone surpassing all previous case numbers. Dengue, which re-emerged in 2000 with DEN-3 as the predominant serotype, has shown notable shifts in serotype dominance over time. From 2015 to 2018, DEN-2 was the dominant strain, followed by a resurgence of DEN-3 from 2019 to 2022. In 2023, DEN-2 again became predominant, likely contributing to the unprecedented severity of the outbreak.[3–6] The shift from DEN-3 (2019–2022) to DEN-2 in 2023 likely contributed to the severe dengue outbreak in Bangladesh due to antibody-dependent enhancement (ADE). Individuals previously infected with DEN-3 may have developed more severe disease upon secondary infection with DEN-2, as non-neutralising antibodies can enhance viral entry and immune response, increasing disease severity.[29,30] This, along with other factors like climate change and increased mosquito activity, likely drove the record-high cases and fatalities in 2023.

Serotyping analysis in this study identified DEN-2 as the predominant serotype in both NS1-positive (97.5%) and NS1-negative (84%) cases, with DEN-3 detected in 16% of NS1-negative cases. This finding aligns with several studies conducted in Bangladesh in 2023, which similarly reported the predominance of DEN-2.[6,31,32] This serotype variation likely contributes to differences in NS1 sensitivity. For example, Fry and coworkers reported that DEN-2 exhibits lower NS1 detection rates compared to other serotypes, a trend reflected in the current findings.[21] Additionally, the presence of secondary infections further reduces NS1 detectability, as pre-existing IgG antibodies can form immune complexes with NS1 antigen, facilitating its clearance. This phenomenon, observed in our study, aligns with findings by Guzmán et al, emphasising the interplay of pre-existing antibodies in secondary infections and their impact on diagnostic accuracy.[22]

The day-wise analysis of IgM and IgG positivity in NS1-negative but RT-PCR-positive cases revealed that IgM antibodies became detectable around day 5 of fever, with a significant increase by days 6 and 7. Conversely, IgG

antibodies, indicative of secondary infections, were detectable in some cases as early as day 2 of onset. This pattern corroborates findings from Vázquez et al highlighting the utility of IgG detection as a marker of secondary Dengue infections, particularly when NS1 results are negative. In Dengue cases, the presence of NS1 along with IgM or IgG helps distinguish primary from secondary infections. IgM with NS1 typically indicates primary infection, while IgG with NS1 in the early days of fever suggests secondary infection. However, in secondary cases, pre-existing neutralising antibodies can clear NS1 antigen rapidly, leaving early IgG as the sole marker of active infection. This can be misinterpreted as a past infection, necessitating further testing to confirm the acute phase and identify patients at higher risk of severe outcomes.[33] The deadly 2023 Dengue outbreak in Bangladesh highlights the critical need for early, accurate diagnosis and timely intervention to prevent severe cases and reduce mortality.

Limitations

This study's limitations include a relatively small sample size, particularly in the NS1-negative group, which may limit the generalizability of the findings. Additionally, the study could benefit from controlling for confounding factors and incorporating more comprehensive diagnostic methods for better accuracy and broader applicability. Despite the robustness of study findings, the use of different NS1 rapid card test kits might cause outcome bias; however, our study objectives in dengue outbreaks was to highlight that NS1 test sensitivity to reinforcing a key message that no one should rely solely on a negative NS1 result to rule out dengue, regardless the types of kit was used. Future studies should include a larger, more diverse sample size and account for potential confounding factors to enhance the reliability and generalizability of the findings.

Conclusion

Despite moderate sensitivity and specificity compared to the RT-PCR test, the NS1 rapid test remains a valuable tool for early Dengue diagnosis, particularly in NS1-negative cases or suspected secondary infections. The inclusion of RT-PCR and antibody profiling can enhance diagnostic accuracy and provide critical epidemiological insights, essential for effective outbreak management.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The lead author Dr. Ruksana Raihan had full access to all of the data in this study and took complete responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

We would like to express our sincere gratitude to US Bangla Medical College, Ahsania Mission Cancer and General Hospital, the Society for Medical Virologists, and Lab Quest Molecular Lab for their valuable support and collaboration throughout this research.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

There is no funding to report.

Disclosure

The authors declare no conflicts of interest in this work.

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