COVID-19 Antibodies as Predictor of Severe Dengue among Hospitalized Children with Dengue Illness in the Post-third-wave Period of COVID-19 Infection in India

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ABSTRACT
Background: There were widespread unconfirmed reports about the increased severity of dengue post-second wave of the COVID-19 pandemic in India. It is known that a second dengue infection with a different strain in an individual can trigger antibody-dependent enhancement (ADE). A similar phenomenon is hypothesized for severe COVID-19 infection since both dengue and COVID-19 are viral diseases with different and varying strains. However, much research is needed to confirm this hypothesis. In this context, we intended to assess the severity of dengue illness in relation to previous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, possibly the role of COVID-19 antibodies as an early predictor of severe dengue illness.

Objective: To assess the utility of COVID-19 antibodies for early identification of severe dengue illness among children in the post-third-wave period of COVID-19 infection in India.

Materials and methods: All hospitalized children with dengue illness were categorized as severe (shock and/or hemorrhage and/or multi-organ dysfunction) and non-severe dengue illness (dengue with or without warning signs) as per WHO definition. COVID-19 antibody titers were estimated in both groups. Clinical features and seroprevalence of COVID-19 antibodies were compared in both groups.

Result: A total of 31 children were studied (13 severe and 18 non-severe dengue illnesses). The most common symptoms prior to presenting to the hospital included fever (100% in both groups), vomiting (85% in severe and 63% in non-severe), abdominal pain (85% in severe and 50% in non-severe), poor feeding (54% in severe and 28% in non-severe), and skin rashes (15% in severe and none in non-severe). The mean duration from the onset of fever to the first hospital visit was 4.6 days in severe illness and 5.3 days in non-severe dengue illness. The mean duration of hospitalization was 9.7 days in severe dengue illness and 4.1 days in non-severe dengue illness. While 92.3% of all severe dengue had significantly higher COVID-19 antibody titers, it was found elevated only in 44.4% of the children with non-severe dengue illness (p-value 0.0059; Yates’ corrected p-value 0.0179).

Conclusion: Clinical symptoms prior to presenting to the hospital were fever, vomiting, abdominal pain, poor oral feeding, and skin rashes. While fever, vomiting, and abdominal pain were seen commonly in both severe and non-severe dengue illnesses, the presence of skin rash during febrile phase is associated with severe dengue illness only. Hospitalized children having severe dengue had increased seroprevalence of COVID-19 antibodies (92.3%) compared to children with non-severe dengue (44.4%). However, there is no correlation of the severity of dengue illness with absolute values of COVID-19 antibody levels. Therefore, the presence of COVID-19 antibodies (previous COVID-19 infection) can be a predictor of severe illness in children with dengue especially if associated with poor oral feeding and skin rashes. The limitation of the study is its lesser sample size to conclude any definitive statement; nevertheless, the study paves way for a similar cohort of a larger sample size to draw conclusions.

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INTRODUCTION
In 2019, WHO identified dengue as one of the four main infectious diseases threatening global health.1 The initial dengue outbreaks in India were documented way back in 1946 and gradually by 1968, the outbreaks were more frequently prevalent spreading from the Eastern coast of India to Northern and Central India.2 Epidemics of dengue in urban Indian society are perennial and the proportion of dengue cases is only seeing an increase in recent years.

There were widespread unconfirmed reports about the increased severity of dengue post-second wave of the COVID-19 pandemic in India. It is known that a second dengue infection with a different strain in an individual can trigger ADE.3 A similar phenomenon is hypothesized for severe COVID-19 infection since both dengue and COVID-19 are viral diseases with different and varying strains.3 However, much research is needed to confirm this hypothesis. In this context, we intended to assess the severity of dengue illness in relation to previous SARS-CoV-2 infection.

Children less than 14 years of age across the globe including India were not vaccinated against SARS-CoV-2 and, hence, detection of significant SARS-CoV-2 antibody titers would infer a previous natural COVID-19 infection. In our study, we assessed the SARS-CoV-2 antibody titers among all hospitalized children with dengue illness. We then compared the clinical features and presence of SARS-CoV-2 antibodies between severe dengue and non-severe dengue illness. Our study aims to support or refute the claim that previous COVID-19 infection is associated with increased severity of dengue illness.

The results of our study can pave way for further research on the association of previous COVID-19 infection to the severity of dengue illness. Further, our study would help in the early identification of severe dengue illness among children in the post-third-wave period of COVID-19 infection in India.

MATERIALS AND METHODS
Place of Study
The study was conducted in the Department of Pediatrics in a tertiary care hospital setting in New Delhi from October to November 2021.

Study Design
A prospective observational study.

1Graded Specialist, Department of Pediatrics, Base Hospital Delhi Cantonment; 2Classified Specialist, Department of Medicine, Air Force Central Medical Establishment; 3Senior Advisor; 4Classified Specialist; 5Junior Resident, Department of Pediatrics, Base Hospital Delhi Cantonment, New Delhi, Delhi, India; *Corresponding Author

Sample Size
A total of 31 children who were admitted in the hospital following dengue illness were included in the study.

Methodology
All children of consenting parents less than 14 years of age who met all the inclusion and exclusion criteria were included in the study. All children were screened for active COVID-19 infection by reverse transcription-polymerase chain reaction (RT-PCR) for nCoV before admission. Only children who were negative for COVID-19 infection were included in the study.

Admitted children were classified into two groups namely severe dengue and non-severe dengue as per WHO classification.4 Severe dengue comprised those children who had clinical evidence of severe plasma leakage manifesting as shock/respiratory distress or hemorrhage or multi-organ dysfunction. Non-severe dengue included children with lab-confirmed dengue with or without warning signs.

Epidemiological parameters, clinical manifestations, and levels of SARS-CoV-2 antibodies were compared between the two groups and analyzed. Possible predictors of severe dengue are identified.

Inclusion Criteria
- Children less than 14 years of age with lab diagnosis of dengue (NS1Ag positive).
- Requiring in-patient care for dengue illness.
- No previous congenital or acquired comorbid illness.
- Negative RT-PCR for nCoV.

Exclusion Criteria
- Individuals not meeting the inclusion criteria.
- Children of non-consenting parents.

RESULT
A total of 31 children (16 males and 15 females) who were hospitalized with dengue illness were subjected to this study. Hospitalized children were classified into two comparison groups, namely severe dengue and non-severe dengue (uncomplicated dengue) as per WHO classification. The age of children in this study ranged from 1 month to 14 years. The mean age in the severe group was 8.4 years and in the non-severe group was 4.6 years (Table 1). About 80% of all admitted children with dengue were under 5 years. There were no underlying chronic medical conditions in any of the children in both groups since children with underlying diseases or disabilities were excluded from the study. The mean duration from the onset of fever to the first hospital visit was 4.6 days in the severe group and 5.3 days in the non-severe group (Table 1).

Among the children who were hospitalized with dengue, all of them had fever (n = 31/31) in both groups. Apart from fever, the other common symptoms prior to the hospital visit included vomiting, abdominal pain, poor oral feeding, and skin rashes. While 84.6% of children with severe dengue had vomiting, 62.5% of children with non-severe dengue had the same. Similarly, abdominal pain and poor oral feeding were seen in 84.6 and 53.8%, respectively, in children with severe dengue and in 50 and 28%, respectively, in children with non-severe dengue (Table 1). Also, 61.5% (8/13) of children with severe dengue had vomiting, abdominal pain, and poor oral feeding while only 33.9% (7/18) of children with non-severe dengue had all three symptoms. About 15.4% (2/13) of children with severe dengue alone exhibited skin rashes during the febrile phase, while none of the children hospitalized for non-severe dengue had any skin rashes during the febrile phase of illness. Among the children hospitalized for severe dengue, the most common form of severe presentation was the presence of shock on arrival to the hospital; seen in 53.8% of severe dengue (n = 7).

While 92.3% (n = 12) children with severe dengue had significant levels of SARS-CoV-2 antibody levels indicating a previous COVID-19 infection, only 44.4% (n = 8) of children with non-severe dengue had evidence of previous COVID-19 infection (p-value 0.0059, Yates’ corrected p-value 0.1789) (Table 2). The mean total SARS-CoV-2 antibody level in children with severe dengue was 74.3 AU/mL as compared to 34.9 AU/mL in the non-severe group. The decreased level of mean total SARS-CoV-2 antibody level in children with non-severe dengue is due to a lower incidence of previous COVID-19 infection in the non-severe group. However, there is no correlation of the severity of dengue illness with absolute values of COVID-19 antibody levels. The mean total SARS-CoV-2 antibody level among the children with previous COVID-19 infection, that is, in children with a significant rise in COVID-19 antibody level (above lab threshold

Table 1: Epidemiological and clinical features of severe and non-severe dengue illness

<table>
<thead>
<tr>
<th></th>
<th>Severe dengue (n = 13)</th>
<th>Non-severe dengue (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>8.4</td>
<td>4.6</td>
</tr>
<tr>
<td>Gender (male/female) (n)</td>
<td>6/7</td>
<td>10/8</td>
</tr>
<tr>
<td>Underlying medical condition(s)</td>
<td>Nil significant</td>
<td>Nil significant</td>
</tr>
<tr>
<td>Average duration from fever onset to the first hospital visit (days)</td>
<td>4.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Clinical signs and symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vomiting (%)</td>
<td>84.6</td>
<td>62.5</td>
</tr>
<tr>
<td>Abdominal pain (%)</td>
<td>84.6</td>
<td>50</td>
</tr>
<tr>
<td>Poor oral feeding (%)</td>
<td>53.8</td>
<td>28</td>
</tr>
<tr>
<td>Skin rashes (%)</td>
<td>15.4</td>
<td>None</td>
</tr>
<tr>
<td>Presence of shock on arrival to the hospital (%)</td>
<td>53.8</td>
<td>–</td>
</tr>
<tr>
<td>RT-PCR for SARS-CoV-2</td>
<td>Negative in all</td>
<td>Negative in all</td>
</tr>
<tr>
<td>Average total SARS-CoV-2 antibody (IgG + IgM) (normal &lt; 15 AU/mL)</td>
<td>74.3 (7.8–205.2)</td>
<td>34.9 (4.1–112)</td>
</tr>
<tr>
<td>Serology for dengue/malaria/scrub typhus/leptospirosis/chikungunya</td>
<td>NS1Ag positive in all</td>
<td>NS1Ag positive in all</td>
</tr>
<tr>
<td>Average length of hospital stay (days)</td>
<td>9.7 days</td>
<td>4.1 days</td>
</tr>
</tbody>
</table>

Table 2: Chi-square test

<table>
<thead>
<tr>
<th></th>
<th>COVID antibodies positive</th>
<th>COVID antibodies negative</th>
<th>Marginal row totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe dengue</td>
<td>12</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Non-severe dengue</td>
<td>8</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Marginal column totals</td>
<td>20</td>
<td>11</td>
<td>31 (grand total)</td>
</tr>
</tbody>
</table>

The Chi-square statistic is 7.554. The p-value is 0.0059 (significant p < 0.5); The Chi-square statistic with Yates’ correction is 5.608. The p-value is 0.0059 (significant p < 0.5)
for determining positivity) is similar in both groups (63.68 AU/mL in the severe group and 61.1 AU/mL in the non-severe group), thereby indicating that there is no correlation of the severity of dengue illness with absolute values of COVID-19 antibody levels. The average duration of hospital stay was obviously higher in the severe dengue group; 9.7 days in the severe group compared to 4.1 days in the non-severe group (Table 1).

**Discussion**

COVID-19 pandemic and recurrent dengue epidemics have not only exhausted the already fragile health system in densely populated tropical countries but also have turned into a global threat. While the coronavirus continues to mutate into different variants creating subsequent COVID-19 pandemic waves, the disease is far from over. Dengue, an illness age immemorial, continues to haunt the human race even in this pandemic period. There are numerous newspaper reports claiming an increased number and severity of dengue illness post-second wave of the COVID-19 pandemic; the Indian capital city Delhi recorded 9,545 dengue cases and 23 fatalities in 2021 compared to 1,072 cases with one death in 2020, 2,036 cases with two deaths in 2019, and 2,798 cases with four deaths in 2018.5-7

Dengue viruses belong to the Flaviviridae family and have four serotypes namely DENV-1, DENV-2, DENV-3, and DENV-4.8 WHO 2009 dengue guidelines categorized dengue illness into severe and non-severe dengue.9 For practical reasons, WHO recommends further splitting of the larger non-severe group into two subgroups—patients with warning signs and those without them.4 The criteria for diagnosis of severe dengue include (a) severe plasma leakage manifesting as shock or respiratory distress and/or (b) severe bleeding and/or (c) severe organ involvement. Warning signs of dengue include (a) abdominal pain or tenderness, (b) persistent vomiting, (c) clinical fluid accumulation, (d) mucosal bleed, (e) lethargy, restlessness, (f) liver enlargement >2 cm, and (g) increase in hematocrit concurrent with a rapid decrease in platelet count (adapted from WHO dengue: guidelines for diagnosis, treatment, prevention and control, 2009).

In the vast majority of the individuals affected by the dengue virus, dengue fever presents as a mild illness only. The symptoms include fever, headache, abdominal pain, and nausea. The activation of local dendritic cells and macrophages following virus inoculation by the Aedes mosquito and subsequent entry into the bloodstream results in leukopenia and thrombocytopenia.9 Both humoral and cell-mediated immune responses are mounted resulting in the elimination of the dengue virus. Humoral immunity is responsible for the production of serotype-specific antibodies. These antibodies do not neutralize other dengue virus serotypes, thereby offering no protection against them but, however, cross-reacts.10 A subsequent infection with another dengue serotype is more severe than the previous infection due to the presence of these non-neutralizing cross-reacting pre-existing antibodies.11 The binding of suboptimal antibodies to the cell surface increases the entry of the virus into host cells, resulting in increased viral load and possibly severe disease.12 The enhanced severe dengue illness manifests as dengue hemorrhagic fever and dengue shock syndrome.13

A similar mechanism of ADE is proposed for severe COVID-19 infection.14 Coronavirus are members of the Coronaviridae family and like dengue viruses, they too are RNA viruses. The classical spike (S) protein of coronavirus binds to the enzymatic domain of the angiotensin-converting enzyme 2 on the host cell surface. The hydrolyzation of S protein paves entry for the coronavirus inside the host cell.15

However, the presence of SARS-CoV-2 antibodies facilitating ADE for severe dengue illness is not postulated so far. Though both are different viruses belonging to different families, their genetic makeup is similar (RNA viruses). Further, ADE-mediated severe second infection is observed in both diseases. Also, COVID-19 and dengue coinfection was associated with severe disease and fatal outcomes.16 While infection with SARS-CoV and Middle East respiratory syndrome–related coronavirus (MERS-CoV) were limited to certain geographical areas, the SARS-CoV-2 pandemic swept across the globe sparing no country. Hence, the presence of SARS-CoV-2 antibody is much more common among the general population than in previous coronavirus epidemics. There could be a possible reaction of these pre-existing COVID-19 antibodies facilitating the entry of dengue virus into host cells thereby causing increased viral load and severe disease.

Also, we are now aware of the multisystem inflammatory syndrome in children (MIS-C) and Multisystem Inflammatory Syndrome in Adults (MIS-A, respectively). The level of SARS-CoV-2 antibodies drastically decreased after 4 months of natural infection, more gradually over the next 7 months, and persist up to 11 months.17 Multisystem inflammation is encountered in this period where the antibody titers are high.18 The Morbidity and Mortality Weekly Report (MMWR) criteria for MIS-A mandate positive test for current or previous SARS-CoV-2 infection (nucleic acid, antigen, or antibody) during admission or in the previous 12 weeks.19 Another possible reason for the increased severity of dengue in the immediate post-second-wave period of COVID-19 could be due to the increased pro-inflammatory condition in the host because of COVID-19 infection.

The reason for the increased severity of dengue illness in the aftermath of COVID-19 waves may be multifactorial and possible causes can only be hypothesized at this stage; a couple of which are discussed above. However, if there could be an association established with the presence of SARS-CoV-2 antibodies (previous and recent COVID-19 infection) to severe dengue illness, we can better anticipate and prepare ourselves for severe dengue illness so as to decrease morbidity and mortality. Since many variants of COVID-19 are emerging and countries reporting a fresh surge in COVID-19 cases, the presence of COVID-19 antibodies can be a reliable marker to predict severe dengue illness.

In this pretext, we estimated the prevalence of SARS-CoV-2 antibodies among hospitalized children with dengue illness. The reason for choosing the pediatric population is that the presence of significant levels of SARS-CoV-2 antibodies in children less than 14 years would mean a previous natural infection since this age group has not been vaccinated so far.

Our results suggested that significantly raised levels of SARS-CoV-2 antibodies were found in 92.3% of children with severe dengue when compared to only 44.4% of children with non-severe dengue (p-value 0.0059, Yates’ corrected p-value 0.1789). However, there is no correlation of the severity of dengue illness with absolute values of COVID-19 antibody levels. While a child with a COVID-19 antibody level of 112 AU/mL had non-severe dengue, another child with a COVID-19 antibody level of only 22.3 AU/mL had severe dengue illness. Total SARS-CoV-2 antibody level among children with a significant rise in COVID-19 antibody level (i.e., above lab cut-off value for determining positivity) is similar in both groups—63.68 AU/mL in the severe group and 61.1 AU/mL in the non-severe group. Our study observed a lower mean total SARS-CoV-2 antibody level in the non-severe group as compared to the severe group which is due to a lower incidence of previous COVID-19 infection in the non-severe group.

When the clinical features are compared between the severe and non-severe groups, both had a similar presentation. Fever, abdominal pain, vomiting, and poor oral feeding were observed equally in both
groups. However, the presence of skin rash during the febrile illness was exclusively seen only in the severe group. MIS-C presents similarly with fever, skin rash, and lab evidence of inflammation and multisystem involvement along with a rise in SARS-CoV-2 antibody titers.²⁰ However, dengue NS1 antigen is negative in such cases. In fact, both the cases which presented with skin rashes during the febrile period and treated for severe dengue were managed in-lines on MIS-C since it could be an MIS-C and dengue overlap.

The strength of the study is its novelty and possibly the only study from the Indian subcontinent to draw the utility of COVID-19 antibodies as a useful marker for early identification of severe dengue.

The major limitation of the study is the lesser sample size of admitted dengue patients since the study was conceived in the middle of the dengue epidemic which was soon over after the onset of winter in India. However, the available results that may make a huge difference are the management of dengue, the epidemic of which has already begun in many Indian states.

**Ethics Approval and Consent to Participate**

Ethics approval was obtained from the Institute Ethics Committee. Verbal consent was obtained from all parents of the children participating in the study.

**Conclusion**

Owing to the available smaller sample size of the study, the study is not powered to make any definitive conclusions. The results, however, convey that the presence of COVID-19 antibodies predicts the severity among hospitalized children with dengue illness. However, there is no correlation of the severity of dengue illness with absolute values of COVID-19 antibody levels.

Also, the common clinical symptoms of dengue prior to presenting to the hospital were fever, vomiting, abdominal pain, poor oral feeding, and skin rashes. While fever, vomiting, and abdominal pain were seen commonly in both severe and non-severe dengue illnesses, the appearance of skin rash during the febrile phase is associated with severe dengue illness only.

Therefore, the presence of COVID-19 antibodies (previous COVID-19 infection) can be a predictor of early severe dengue among hospitalized children with dengue illness, especially if associated with skin rashes during the febrile phase.

Though the present study is not powered to make any recommendations, the results clearly indicate the need for further studies powered to make recommendations over the routine estimation of COVID-19 antibodies among hospitalized children with dengue illness.

**References**

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