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Clinical implications of Widal false positivity among children presenting with dengue infection: a comparative cohort study

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ABSTRACT:

Objective: Dengue fever often coincides with other endemic infections such as typhoid, leading to diagnostic overlap. The Widal test, though frequently used for typhoid diagnosis, may show false positivity in dengue cases due to nonspecific immune activation. However, the clinical implications of Widal test results in children with confirmed dengue without typhoid co-infection remain unclear.

Patients and Methods: This prospective comparative cohort study was conducted at a tertiary care pediatric hospital in North India between June 2022 and November 2023. Children aged 1 month to 18 years with confirmed dengue infection were enrolled. Widal positivity was defined as TO (Typhi O; somatic antigen of *Salmonella Typhi*) or TH (Typhi H; flagellar antigen of *Salmonella Typhi*) titers > 1:80. Blood cultures were performed in all patients to exclude typhoid fever. Baseline clinical parameters, hematological and biochemical markers, and clinical outcomes were compared between Widal-positive and Widal-negative groups.

Results: Out of 152 enrolled children with confirmed dengue, 60 (39.5%) were Widal-positive and 92 (60.5%) Widal-negative. Children with Widal-negative dengue had significantly higher blood urea levels (p=0.02), prolonged prothrombin time (p=0.01), and greater need for fluid boluses (17.4% *vs.* 1.7%, p=0.003), suggesting a more severe disease profile, although these findings represent exploratory outcomes and should be interpreted as hypothesis-generating. In contrast, Widal-positive children had a longer duration of fever (p<0.001) and a higher frequency of vomiting, but did not show increased complication rates.

Conclusions: In this exploratory analysis, Widal negativity in children with dengue infection appears to be associated with more severe clinical manifestations. While Widal positivity is often disregarded as nonspecific, its absence may carry prognostic significance. As severe dengue was an exploratory outcome, these findings warrant further investigation and should be considered hypothesis-generating.

Keywords: Dengue, Widal, Typhoid, Capillary leak syndrome, Fluid resuscitation, Disease severity.

INTRODUCTION

Dengue remains prevalent across a wide region of the world and continues to pose a threat to populations with severe morbidities and mortality associated with its severe forms[1,2]. Worldwide, there have been extensive efforts to identify parameters during the early stages of illness that are able

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to reasonably predict the severe clinical course ahead[3-5].

In most endemic areas, dengue occurs during the time of the year, which is also the peak season for other tropical infections like malaria, typhoid, hepatitis A, and scrub typhus. Often, these infections are considered to be a causative factor behind every acute febrile illness presenting during this season.

It is not unusual to find Widal (false) positivity among children admitted to the hospital with proven dengue infection. While it may be assumed to result from the generally poor specificity of the Widal test, only a proportion of those infected with dengue show false positivity in this serological test. The Widal test is a tube or slide agglutination test that detects antibodies against the somatic (O) and flagellar (H) antigens of *Salmonella Typhi* and *Paratyphi*. Although simple and inexpensive, it suffers from several important limitations, such as cross-reactivity with other infections, persistence of antibodies from past exposure or vaccination, and poor specificity in endemic regions[6,7]. The pathophysiological mechanisms in dengue infection are known to involve several complex antigen-antibody interactions leading to inflammatory responses responsible for certain life-threatening manifestations[8-10]. Hence, this study was undertaken to explore whether the result of the Widal test carries any significance in the context of the clinical course and outcomes among children admitted with dengue.

PATIENTS AND METHODS

This was a comparative cohort study conducted at a tertiary care teaching hospital in the north Indian region over a period of 18 months between June 2022 and November 2023. Children aged one month to 18 years admitted to the Department of Pediatrics with confirmed dengue fever [clinical features of dengue with non-structural protein 1 (NS1) antigen or dengue immunoglobulin M (IgM) positivity] were offered participation and those willing were prospectively enrolled after obtaining informed consent. Children with chronic liver, kidney, or lung disease, or those with known immunodeficiency disorders, were excluded. Children who had enteric fever in the last 6 months, who were culture-positive for enteric fever in the current illness, or who had another associated infection, such as malaria, were excluded from the study. The study proposal was approved by the Institutional Ethics Committee (2022-06-IM-30, dated 25-06-2022).

As a standard practice in our department, all children presenting with acute febrile illness without any overt localising features are screened during their first evaluation for a set of tropical infections (dengue, malaria, typhoid, scrub typhus, etc.) which are simultaneously prevalent during the second half of the year in the region. Hence, the Widal test report was available for all children who tested positive for dengue. In all the enrolled participants, classification and management of dengue were done in accordance with the standard of care[11,12]. Baseline demographic data and clinical course details were recorded on a pre-structured proforma. Weight Z-scores and BMI Z-scores were calculated using WHO growth standards, where Z-scores represent standard deviation units from the median of the reference population. Blood samples collected at presentation were tested for hematological parameters, liver and kidney function tests, Widal test, blood culture and blood smear for malaria parasite. Widal positivity was defined as >1:80 titers of TO (Typhi O; somatic antigen of *Salmonella Typhi*) or TH (Typhi H; flagellar antigen of *Salmonella Typhi*)[13]. Thrombocytopenia was defined as platelet counts $<1.5 \times 10^5/mm^3$ and severe thrombocytopenia as $<0.5 \times 10^5/mm^3$. Total leukocyte counts of $<4 \times 10^3/mm^3$ were defined as leukopenia. Elevated transaminases were defined as Serum Glutamic Oxaloacetic Transaminase (SGOT) or Serum Glutamic Pyruvic Transaminase (SGPT) >2 times the upper limit of normal for age and gender. The outcome parameters included duration of fever (calculated as total days of fever from onset as reported by parents until defervescence during hospitalization), platelet counts, change in hematocrit, the need for blood product transfusion, bleeding episodes, oxygen support, hypotension, need for fluid boluses (rapid IV resuscitation for hemodynamic compromise), duration of intravenous fluids (maintenance and replacement therapy during hospitalization until adequate oral intake was established), requirement of inotropic support, need of mechanical ventilation and mortality.

Dengue NS1 antigen was detected by dengue NS-1 enzyme-linked immunosorbent assay (ELISA) kit by Oscar Medicare Pvt. Ltd. (New Delhi, India) and Dengue IgM antibodies were detected by Dengue IgM Capture ELISA Kit by the Indian Council of Medical Research – National Institute of Virology, Pune (India). Widal test was done by TYDAL, a Widal slide and tube agglutination test that detects the presence of the serum agglutinins (O, H) in the patient's serum, manufactured by Tulip Diagnostics (P) Ltd (Goa, India).

In the absence of reference data, the sample size was calculated assuming the difference in incidence of severe thrombocytopenia between Widal-positive and Widal-negative dengue fever to be 25%. With a power of 80%, an alpha error of 5% and a confidence interval of 95%, a minimum sample size of 120 (60 in each group) was required. Severe thrombocytopenia, therefore, represented the prespecified primary outcome used solely for sample size estimation. All other clinical and biochemical parameters, including severe dengue, fluid bolus requirement, and biochemical derangements, were defined *a priori* as exploratory or secondary outcomes.

Statistical Analysis

Data were entered in Excel and analyzed using Statistical Package for Social Sciences (SPSS) software (IBM Corp, Armonk, NY, USA) version 25.0. Mean and standard deviation were calculated for normally distributed data and median and interquartile ranges were calculated for non-normally distributed data. Data normality was checked by using the Shapiro-Wilk test. Chi-square/Fisher's exact tests were applied for comparison of categorical variables. Student's *t*-test was used for comparison of continuous variables with normal distribution and for non-normally distributed data, the Mann-Whitney U test was performed. Pearson and Spearman correlation coefficients were used for assessing the correlation between continuous variables. To minimize inflation of the type I error from multiple comparisons, all study variables were categorized into descriptive (baseline and demographic), exploratory (clinical presentation), and inferential (outcome-related) groups. Formal hypothesis testing was confined to key outcome variables, while other comparisons were treated as descriptive summaries. Univariable logistic regression was first performed to evaluate the unadjusted association between Widal status and severe dengue. To prevent overfitting, given the limited number of severe dengue events, covariates for the multivariable model were selected using both clinical relevance and a liberal univariable screening threshold ($p<0.40$), consistent with recommended approaches for small-sample logistic regression. Based on this strategy, the final multivariable model included three predictors: Widal status, baseline platelet count, and symptom duration at presentation. Age and gender were excluded to maintain an acceptable events-per-variable ratio and ensure model parsimony. A p-value of <0.05 was considered significant. To avoid misinterpretation, we explicitly distinguished between primary and exploratory outcomes. Severe thrombocytopenia remained the only prespecified primary endpoint. Severe dengue and fluid bolus requirement were analyzed as exploratory outcomes, and results related to these variables are interpreted as hypothesis-generating rather than confirmatory.

A completed STROBE checklist has been provided as Supplementary File 1 to ensure comprehensive and transparent reporting of this observational study.

RESULTS

A total of 159 children were admitted with confirmed dengue fever during the study period. After excluding two children with blood culture-positive enteric fever, three children with underlying congenital heart disease, and two children with associated malaria, 152 children with a median (Q1-Q3) age of 7 (4-11.25) were included. All study variables were complete with no missing data. Of these, 60 children tested positive for Widal, while the remaining 92 were Widal negative. Among the 60 Widal-positive children, the distribution of titres was: TO-only 71.7%, TH-only 5%, and both TO and TH 23.3%.

The baseline demographic parameters and clinical features are compared among the groups in Table 1. Most parameters did not show any significant difference except for fever and vomiting at presentation ($p=0.001$ and $p=0.03$, respectively), which are more likely to be seen among children with positive Widal test. Only one participant among the Widal-negative group reported a history of previous dengue infection, while none reported it among the Widal-positive group. The dengue serotype of the previous infection was not known in the patient. The baseline hematological and biochemical parameters are compared among the groups in Table 2. The comparison of clinical course and outcome parameters among the groups is depicted in Table 3. It should be noted that 'duration of IV fluids' refers to maintenance

Table 1. Comparison of demographic and clinical characteristics among the Widal-positive and Widal-negative groups at presentation.

Parameter	Overall (N=152)	Widal-positive (N=60)	Widal-negative (N=92)	*p*-value
Age (years) [Median (Q1-Q3)]	7 (4-11.25)	8 (5-12)	6 (3-10.25)	0.31
Male, n (%)	107 (70.4)	42 (70.0)	65 (70.7)	0.93
Weight Z score [Median (Q1-Q3)]	-0.34 (-1.24 to 0.33)	-0.44 (-1.14 to 0.09)	-0.27 (-1.27 to 0.52)	0.5
Body Mass Index Z score [Median (Q1-Q3)]	-0.33 (-1.42 to 0.61)	-0.55 (-1.34 to 0.43)	-0.17 (-1.52 to 0.84)	0.66
Fever, n (%)	119 (78.3)	55 (91.7)	64 (69.6)	**0.001**
Vomiting, n (%)	75 (49.3)	36 (60.0)	39 (42.4)	**0.03**
Jaundice, n (%)	1 (0.7)	0 (0)	1 (1.1)	1.0
Rash, n (%)	35 (23.0)	10 (16.7)	25 (27.2)	0.13
Bleeding manifestation, n (%)	27 (17.8)	13 (21.7)	14 (15.2)	0.30
Encephalopathy, n (%)	9 (5.9)	1 (1.7)	8 (8.7)	0.09
Seizures, n (%)	7 (4.6)	1 (1.7)	6 (6.5)	0.24
Pain abdomen, n (%)	62 (40.8)	26 (43.3)	36 (39.1)	0.60
Hepatomegaly, n (%)	44 (28.9)	22 (36.7)	22 (23.9)	0.09
Splenomegaly, n (%)	13 (8.6)	5 (8.3)	8 (8.7)	0.93

Except for outcome parameters, all other comparisons are descriptive; formal hypothesis testing is limited to outcome variables.

Table 2. Comparison of hematological and biochemical parameters among the Widal-positive and Widal-negative groups at presentation.

Parameter	Overall (N=152)	Widal-positive (N=60)	Widal-negative (N=92)	*p*-value
Platelet count, (× 10^5/mm^3) [Median (Q1-Q3)]	0.79 (0.44-1.88)	0.81 (0.53-1.68)	0.78 (0.38-2.09)	0.53
Platelet count <1.5 × 10^5/mm^3, n (%)	116 (76.3)	48 (80)	68 (73.9)	0.38
Hematocrit (%) [Mean (SD)]	36.7 (6.2)	36.7 (5.7)	36.7 (6.6)	0.96
Total leucocyte count, (× 10^3/mm^3) [Median (Q1-Q3)]	6.7 (4.7-9.9)	7.7 (4.9-11.1)	6.2 (4.5-8.9)	0.13
Leucocyte count <4 × 10^3/mm^3, n (%)	28 (18.4)	9 (15.0)	19 (20.6)	0.39
Serum bilirubin (mg/dL) [Median (Q1-Q3)]	0.6 (0.48-0.77)	0.6 (0.5-0.72)	0.6 (0.47-0.80)	0.81
Serum glutamic oxaloacetic transaminase (IU/L) [Median (Q1-Q3)]	77.5 (48-193)	68 (46-181)	84.5 (49-199)	0.49
Serum glutamic pyruvic transaminase (IU/L) [Median (Q1-Q3)]	55 (27-84)	61 (28-78)	51 (27-88)	0.38
Elevated transaminases, n (%)	64 (42.1)	24 (40.0)	40 (43.5)	0.67
Serum creatinine (mg/dL) [Median (Q1-Q3)]	0.6 (0.3-0.8)	0.59 (0.3-0.78)	0.61 (0.3-0.82)	0.62
Blood urea (mg/dL) [Median (Q1-Q3)]	25 (18-31)	23 (17-25)	30 (26-37)	**0.02**
Prothrombin time (seconds) [Median (Q1-Q3)]	18.2 (16.5-23.3)	16.6 (15.1-18.1)	19.1 (17.9-25.9)	**0.01**

Except for outcome parameters, all other comparisons are descriptive; formal hypothesis testing is limited to outcome variables.

Table 3. Comparison of clinical course and outcome parameters between the Widal-positive and Widal-negative groups.

Parameter	Overall (N=152)	Widal-positive (N=60)	Widal-negative (N=92)	*p*-value
Platelet counts <0.5 × 10^5/mm^3, n (%)	64 (42.1)	22 (36.7)	42 (45.6)	0.27
Duration of fever (days) [Median (Q1-Q3)]	6 (5-7)	7 (6-8.5)	5 (4-6)	**<0.001**
Requirement of oxygen support, n (%)	5 (3.3)	1 (1.7)	4 (4.3)	0.65
Duration of IV fluids (days) [Median (Q1-Q3)]	2 (1-3)	2 (1-2.25)	2 (1-3)	0.06
Requirement of fluid boluses, n (%)	17 (11.2)	1 (1.7)	16 (17.4)	**0.003**
Requirement of PRBC transfusion, n (%)	6 (3.9)	3 (5.0)	3 (3.3)	0.68
Requirement of platelet transfusion, n (%)	18 (11.8)	5 (8.3)	13 (14.1)	0.28
Duration of stay (days) [Median (Q1-Q3)]	5 (4-6)	5 (4-5)	5 (4-6)	0.55
Mortality, n (%)	1 (0.7)	0 (0)	1 (1.1)	1.0

Except for outcome parameters, all other comparisons are descriptive; formal hypothesis testing is limited to outcome variables. Packed red blood cell (PRBC).

and replacement therapy provided to all children until adequate oral intake is tolerated, whereas 'fluid boluses' represent additional rapid resuscitation administered to a subset with hemodynamic compromise. Children with positive Widal test experienced fever for a significantly longer time, as depicted in Figure 1.

The Widal Negative group had a significantly higher requirement of fluid boluses during the clinical course (17.4% *vs.* 1.7%, p=0.003). Apart from fluid boluses, only one participant among the Widal-negative group required inotropic support compared with none in the Widal-positive group. The increased need for IV fluid boluses in the Widal-negative group was associated with a higher frequency of clinical instability and capillary leak features at presentation and during the course of illness. Poor feeding (22 *vs.* 7), lethargy (8 *vs.* 1), low pulse volume (5 *vs.* 1), and abdominal distension (3 *vs.* 0) were more common among Widal-negative children. In addition, requirement for oxygen (4 *vs.* 1), inotropic support (1 *vs.* 0), and pleural effusion (6 *vs.* 2) were also observed more frequently in this group. The rest of the parameters, including hematological, biochemical parameters, rate of morbidities, complications, need for various therapeutic interventions, or mortality, did not show any significant difference among the groups.

According to WHO severity criteria[12], severe dengue was observed in 25 children (16.4%) overall, with 4 (6.7%) in the Widal-positive group and 21 (22.8%) in the Widal-negative group (p=0.016). A 2×2 contingency table comparing Widal negativity with the presence of severe dengue is presented in Table 4, along with diagnostic performance indices (sensitivity, specificity, positive predictive value, and negative predictive value). As severe dengue was not a prespecified primary outcome, these observations are exploratory and intended to generate further hypotheses. One child in the Widal-negative group died (survival rate 98.9%), whereas all children in the Widal-positive group survived (100% survival).

On univariable logistic regression, Widal negativity was significantly associated with severe dengue (unadjusted OR: 3.91; 95% CI: 1.18-12.94; p=0.026). In the reduced multivariable logistic regression model, Widal negativity remained independently associated with severe dengue (adjusted OR: 4.21; 95% CI: 1.36-13.08; p=0.013). Platelet count and symptom duration showed expected directional effects,

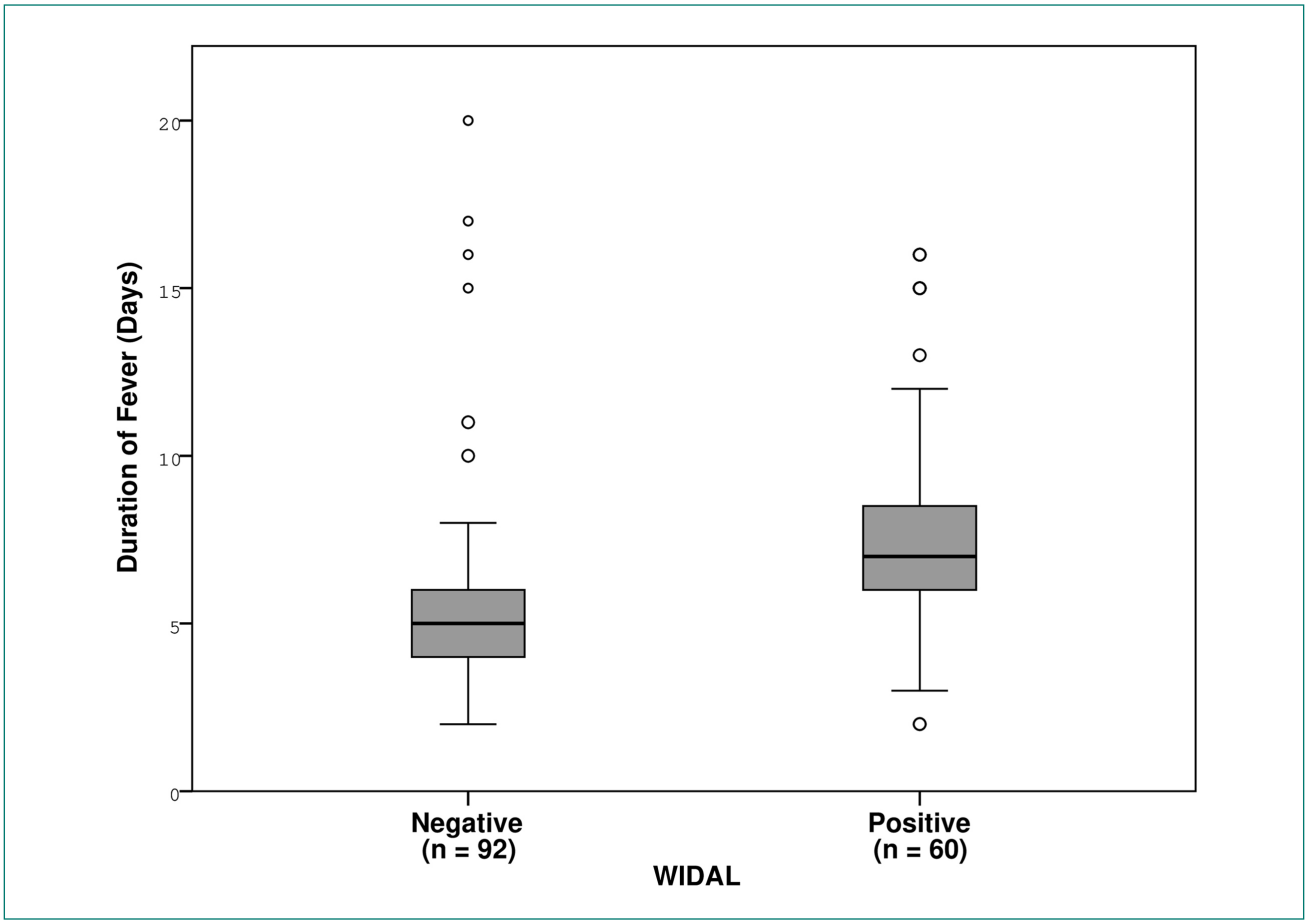

Figure 1. Comparison of duration of fever (days) in dengue infection in Widal-negative vs. Widal-positive groups. Boxplot showing median and interquartile range; whiskers represent 1.5×IQR (Tukey method), and outliers are plotted as individual points.

although only Widal status remained statistically significant in the reduced model. These adjusted associations should be interpreted cautiously, as the possibility of unmeasured confounding cannot be excluded.

As statistical inference was limited to the key outcome parameters, the remaining clinical and biochemical comparisons were considered exploratory, intended to provide descriptive context rather than inferential conclusions.

DISCUSSION

This comparative prospective cohort study, conducted over 18 months, investigated the clinical implications of Widal test positivity in children admitted with dengue infection. A total of 152 children were enrolled, with 92 testing negative for Widal and 60 testing positive. The analysis of complaints at the time of admission revealed that fever and vomiting were significantly more prevalent in the Widal-positive cases. The Widal-negative group exhibited a statistically significant association with a more complicated clinical course. This was notable in the form of coagulopathy, higher requirement of fluid boluses, and derangement in renal parameters. Although the study was powered for thrombocytopenia, exploratory analysis revealed that Widal negativity, rather than being clinically irrelevant, might be associated with markers of more severe disease and could serve as an indirect indicator of systemic involvement in dengue

Table 4. Diagnostic performance of Widal negativity for predicting severe dengue.

Widal Status	Severe dengue (WHO 2009): Yes	Severe dengue (WHO 2009): No	Total
Widal-negative	21	71	92
Widal-positive	4	56	60
Total	25	127	152

Sensitivity: 84.0% (95% CI: 65.3%-93.6%); Specificity: 44.1% (95% CI: 35.8%-52.8%); positive predictive value (PPV): 22.8% (95% CI: 15.4%-32.4%); negative predictive value (NPV): 93.3% (95% CI: 84.1%-97.4%).

infection. Given that only a limited number of outcome parameters were subjected to formal hypothesis testing, the other findings should be viewed as descriptive and hypothesis-generating.

While Widal positivity in dengue patients has often been considered a serological artifact in endemic regions[7,14], we observed that children who were Widal-negative had significantly higher frequencies of adverse clinical and biochemical parameters. Notably, the requirement for fluid boluses was substantially higher among Widal-negative children (17.4% *vs.* 1.7%, p=0.003). These findings suggest that the excess fluid bolus requirement in the Widal-negative group may reflect underlying capillary leak physiology, as evidenced by poor feeding, low pulse volume, pleural effusion, and the need for respiratory or inotropic support, which are well-recognized features of severe dengue.

Additionally, Widal-negative children had significantly elevated levels of blood urea and prolonged prothrombin time compared to their Widal-positive counterparts. These findings suggest greater systemic involvement, including renal hypoperfusion and hepatic dysfunction, which are known complications of severe dengue[10,15,16]. The single mortality in our cohort occurred in a Widal-negative child, while all Widal-positive children survived. This further underscores the potential prognostic implications of Widal negativity. These associations, however, are observational and should not be interpreted as evidence of a causal relationship. They may indicate a pattern worthy of further investigation rather than a direct mechanistic link.

In contrast, children with Widal positivity presented more frequently with vomiting and had longer fever duration (median 7 *vs.* 5 days, p<0.0001) but did not exhibit increased rates of severe complications such as hypotension, bleeding, thrombocytopenia, or multi-organ dysfunction. This dichotomy supports the hypothesis that Widal positivity in dengue may reflect a broader, nonspecific immune activation rather than true coinfection or a worsened disease[7,9,17,18].

Because only 25 children developed severe dengue, the multivariable model was deliberately restricted to a small number of predictors using a liberal univariable screening threshold (p<0.40), consistent with recommended approaches for low-events logistic regression. This strategy minimized overfitting and allowed inclusion of clinically relevant variables. While the association between Widal negativity and severe dengue persisted in this reduced model, the adjusted estimates should still be interpreted with caution. Given the observational nature of the study and the limited number of covariates included, residual or unmeasured confounding could partly account for the observed adjusted association between Widal negativity and severe dengue. In our cohort, 16.4% of children fulfilled the criteria for severe dengue, with a higher proportion in the Widal-negative group (22.8% *vs.* 6.7%). Although numbers were small, this reinforces the observation that Widal negativity could reflect a trend towards more aggressive systemic involvement. The diagnostic performance analysis showed that Widal negativity had a sensitivity of 84% and specificity of 44.1% for identifying severe dengue. Importantly, the high negative predictive value (93.3%) suggests that children with Widal positivity might be less likely to develop severe dengue, adding clinical relevance to the interpretation of Widal results in endemic regions.

Beyond clinical correlations, the association between Widal negativity and disease severity raises the possibility of underlying immunological differences, although this remains speculative and cannot be inferred from the present data. Current literature highlights several immune mechanisms relevant to dengue pathogenesis – including antibody-dependent enhancement (ADE), the role of afucosylated IgG1 in enhancing Fcγ receptor-mediated activation, and the contribution of cross-reactive or heterologous antibodies, which influence disease severity[19-22]. In co-endemic regions, prior exposures to *Salmonella Typhi* or other pathogens may shape baseline immune priming, potentially affecting serological responses such as Widal reactivity. Reports of false-positive typhoid serology in dengue support the idea of cross-reactive or broadly polyclonal immune activation; conversely, the absence of such antibodies (Widal negativity) might indicate a different immunological baseline[17,18,23]. However, these concepts remain hypotheses in the context of our findings. Future studies incorporating serotype data, antibody specificity and glycosylation profiles, and functional assays of ADE are required to determine whether Widal status reflects differences in immune priming or merely coincidental associations.

The Widal test is known to yield false-positive results due to polyclonal B-cell activation, especially in acute viral illnesses like dengue[7,9]. In endemic settings, its utility is further limited by background antibody levels and cross-reactivity. In our study, the relative absence of Widal antibodies in dengue patients appeared to be associated with markers of greater systemic involvement; however, these observations are associative and should be interpreted cautiously. From a clinical perspective, this pattern suggests that Widal test results, although nonspecific, may offer subtle clues regarding disease severity in children with confirmed dengue. While Widal positivity should not be used to initiate anti-typhoid therapy without microbiological confirmation, Widal negativity may warrant closer clinical monitoring. These interpretations remain hypothesis-generating and require validation in larger, multicenter studies.

This study involved the utilization of investigations that are inexpensive, widely available and routinely performed as a part of standard care in children. We performed the analysis using statistical tools

that are widely accepted for such data analysis. These strengths reflect the robust internal validity of the study. Since the study was conducted at an exclusive pediatric teaching institute situated in the national capital region of the country and the state-run institute caters to a wide spectrum of children from various sociodemographic backgrounds coming from the western part of Uttar Pradesh and adjoining regions in the states of Delhi, Haryana and Uttarakhand, the study participants were reasonably representative of the regional population in general.

A major strength of this study is the inclusion of only laboratory-confirmed dengue cases and the systematic use of blood cultures in all patients to rule out enteric fever due to *Salmonella* spp. This approach provided a robust method to exclude active typhoid coinfection. However, it is important to recognize that blood culture, despite being the gold standard, has limited sensitivity – often detecting only 40-80% of typhoid cases – especially in patients who have received prior antibiotics or present late in the illness[6,14]. Therefore, while the absence of *Salmonella* growth in blood cultures strengthens the internal validity of our findings, it does not entirely eliminate the possibility of missed coinfections. Nonetheless, the combined clinical and microbiological screening processes ensured a largely homogenous population for evaluating the clinical implications of Widal test results. Importantly, multivariable logistic regression confirmed that Widal negativity independently predicted severe dengue after adjustment for age, gender, symptom duration, platelet count, and hematocrit. This strengthens the robustness of our findings and supports the potential prognostic value of Widal negativity in the clinical assessment of dengue severity. The dataset had no missing values, which strengthens the completeness of the analyses.

Limitations of the study include its single-center design and the inherent variability of the Widal test, though these were mitigated by standardized testing and interpretation protocols. Although we deliberately focused on inexpensive, routinely performed laboratory parameters in our study, the addition of sophisticated biomarkers such as procalcitonin, high-sensitivity C-reactive protein, cytokines, etc., would certainly enhance the validity of the study's results. Epidemiological details such as history of prior dengue infection, dengue vaccination status, or viral serotype are known to influence immune response and disease severity. These can also potentially provide additional insights into the observed differences between Widal-positive and Widal-negative groups. Future studies incorporating these parameters will help clarify their impact. The exploratory associations with severe dengue were not part of the original power calculations and should be interpreted cautiously. In addition, although blood cultures were performed in all children, false-negative results remain possible, particularly in those who may have received prior antibiotics or presented late in illness. Also, the number of severe dengue events was modest, which constrained the multivariable analysis and required a parsimonious model based on liberal univariable screening thresholds. Despite restricting the number of covariates included, the adjusted model may still be subject to overfitting, and unmeasured confounders such as undocumented antibiotic use, prior dengue infection, or other baseline immune differences may have influenced both Widal reactivity and clinical outcomes.

CONCLUSIONS

In conclusion, this exploratory study suggests that Widal negativity in children with dengue fever could indicate a trend suggesting increased disease severity, as evidenced by greater fluid bolus requirements and more frequent laboratory abnormalities. While Widal testing remains limited as a diagnostic tool for typhoid, its interpretation in the setting of dengue should consider the possibility that a negative result, in the absence of coinfection, could indicate a more aggressive clinical course. All observed associations between Widal negativity and markers of severity should be interpreted cautiously, as the study does not establish causality or underlying mechanisms. Therefore, further hypothesis-driven multicenter research with a larger sample size and the use of sophisticated tools and markers is suggested to explore this novel hypothesis and the underlying immunological mechanism in order to incorporate it into standard care in the management of children with dengue fever, especially in resource-limited settings.

Ethics Approval:
Ethical approval obtained from the Postgraduate Institute of Child Health, Noida, Institutional Ethics Committee (reference No. 2022-06-IM-30, dated 25-06-2022).

Conflict of Interest:
The authors have no competing interests to declare that are relevant to the content of this article.

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K. Saurabh, B.K. Bhakhri and S.B. Mathur contributed to the analysis and interpretation of data.
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All authors contributed to the conception and design of the study, acquisition of data, critical revision of the manuscript, and final approval of the manuscript.

Informed Consent:

Written informed consent was provided by the legal guardians of the participants. Only those who gave consent and were willing to participate were included in the study.

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