Assessing the Serum Chymase Level as an Early Predictor of Dengue Severity

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RESEARCH ARTICLE

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Assessing the serum chymase level as an early predictor of dengue severity

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Abstract

We conducted a prospective, observational study to assess the serum chymase level, a mast cell derived protease, as a predictor of dengue severity. NS1-positive non-severe dengue patients of age ≥14 years with duration of fever ≤4 days were included in the study. At the time of admission, the serum sample was taken for chymase estimation. Patients were followed up to four days after they became afebrile to find out the final diagnosis. Total of 338 non-severe dengue patients were recruited (mean age: 29.15 years; male: 66%). On follow-up, 26 patients (7.8%) developed severe dengue. Only chymase level (adjusted odds ratio [aOR]: 1.787; 95% confidence interval [CI]: 1.309–2.440) and platelet count at admission (aOR: 0.981; 95% CI: 0.968–0.993) were able to predict the severity after adjustment for all variables. But, for prediction of severe dengue, the area under receiver′s operating curve of chymase was 0.835 (95% CI: 0.765–0.905), which was significantly higher than that of the platelet count at admission (0.760, 95% CI: 0.650–0.870) ($p < .001$). Patients who developed severe dengue in due course of illness had significantly higher serum chymase level at admission as compared with the rest of the patients. Similar findings were noted across all age-groups. At an optimum cut-off value of 1.35 ng/ml, chymase had a positive likelihood ratio (LR) of 3.5 and a negative LR of 0.15, for predicting severe dengue. This study demonstrated the potential ability of serum chymase levels at admission, as a biomarker for prediction of severe dengue in due course of illness.

KEYWORDS

biomarker, chymase, dengue, predictors, severe disease

1 | INTRODUCTION

Dengue viral infection is the most common arboviral infection in the world with half of the total population is at risk.[1] Mortality in dengue is related to the fact that disease course from dengue fever (DF) to severe dengue is challenging to predict. It′s a dynamic disease with wide clinical spectrum varying from mild febrile illness to stage of shock and death.[2] For proper triaging of patients, World Health Organization (WHO) in 2009 has proposed a new classification of dengue disease consisting of three categories—dengue without warning signs, dengue with warning signs and severe dengue.[3] Although the disease progression can be predicted based on warning

signs, it requires a strict observation which can be easily missed.[3] The development of warning signs may occur in late-stage, giving a short time for the clinician to judge the protracted course of severe illness. Therefore, there is an unmet need for objective tests which can predict the disease severity well in advance so that appropriate intensive care can be given to a particular subset of patients.[4]

Some biomarkers of dengue severity have been studied recently, including mast cell-derived soluble mediators like tryptase and chymase.[5] It was found that higher levels of serum chymase are present in severe dengue patients than that of non-severe patients.[6] We, therefore, conducted this prospective, single-center, observational study to assess the ability of serum chymase level as an early predictor of dengue severity, among patients presenting with non-severe dengue.

2 | METHODS

2.1 | Study population

This study was a single-center, adult emergency department based, prospective observational study conducted at a tertiary care hospital from 1st August 2018 to 29th February 2020. Acute febrile illness patients were screened for the study for the following criteria: duration of fever for ≤4 days, age ≥14 years, consented to participate (parental consent was obtained for patients of age ≤18 years) and probable DF (definitions available in Supplement). Among the screened patients, only laboratory-confirmed dengue patients (NS1 antigen enzyme-linked immunosorbent assay [ELISA] positive) were included in the study. Patients were excluded if any features of severe dengue (definitions available in Supplement) present at admission; or had a known chronic disease like chronic liver disease and chronic kidney disease. Pregnant patients and patients with a history of atopy were also excluded. Ethical approval was obtained from the Institution Ethics Committee (IEC number: IECPG-262/28.06.2018). Initial diagnosis at admission and final diagnosis after follow-up (till 4 days post-defervescence of fever) were based on the WHO-2009 classification of DF (available in Supplement).[3] Final diagnosis was also classified according to the older classification of dengue by WHO as DF, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) for comparing it with newer WHO-2009 classification.

2.2 | Sample collection, dengue diagnosis, and serum chymase assay

Serum sample of all screened patients was collected for ELISA of dengue NS1 antigen at the time of admission. Patients who were NS1 positive were assayed for serum chymase levels, using Human Mast Cell Chymase ELISA kit, Cusabio (CSB-E13757h) according to the manufacturer's instruction (details available in Supplement).[7]

2.3 | Data collection and study flow

The study flow is depicted in Figure 1. For each included patient, information collected were: demographics—age, gender; clinical signs

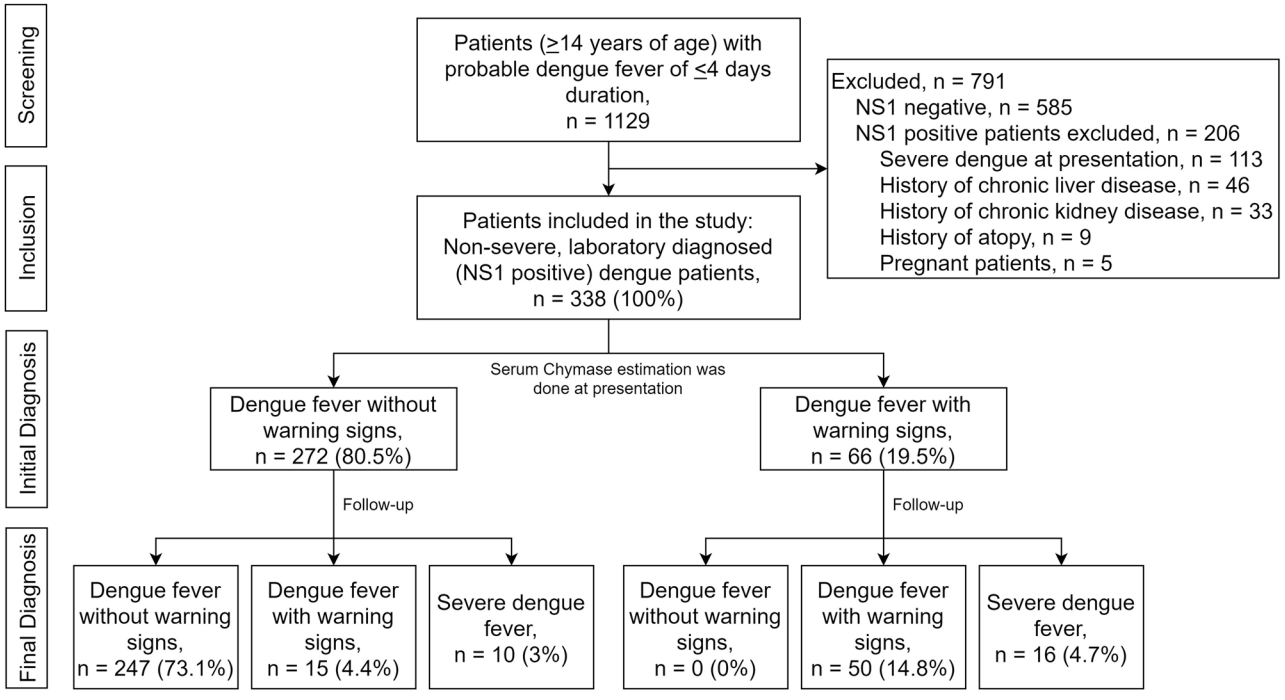

FIGURE 1 Flow diagram showing the study methodology

TABLE 1 Clinical characteristics of the patients included in the study

Characteristics	Final diagnosis, *n* = 338		
	Non-severe dengue		Severe dengue
	Dengue fever without warning signs (DF), *n* = 247	Dengue fever with warning signs (DFWS), *n* = 65	Severe dengue, *n* = 26
Age, mean years (*SD*)	29.5 (12.6)	28.7 (10.6)	26.9 (10.9)
Male gender, *n* (%)	166 (67.2)	44 (67.7)	14 (53.8)
Day of fever at admission, mean (*SD*)	3.4 (0.7)	3.3 (0.8)	3.5 (0.6)
Diagnosis at presentation			
DF, *n* (%)	247 (100)	15 (23.1)	10 (38.5)
DFWS, *n* (%)	0 (0)	50 (76.9)	16 (61.5)
Blood count at admission, mean (*SD*)			
Hemoglobin (in g/dl)	12.8 (2.4)	13.6 (2.0)	12.8 (3.3)
Hematocrit	39.0 (6.1)	40.5 (5.7)	39.0 (9.4)
Platelets (1000 per mm^3)	90.1 (70.1)	63.5 (62.6)	40.2 (52.5)
Total Leukocyte count (1000 per mm^3)	5.3 (2.9)	4.7 (2.6)	4.7 (2.6)
Serum Chymase at admission in ng/ml, mean (*SD*)	1.0 (1.2)	1.2 (1.4)	2.3 (1.1)

Note: % values shown in the cells are column-wise (i.e., final diagnosis-wise).

and symptoms—day of onset of fever, associated symptoms like myalgia, retro-orbital headache, rashes, vomiting, presence of any warning signs, hemorrhagic features, haemodynamic parameters (blood pressure, pulse pressure, capillary refilling time and urine output); laboratory data—total leukocyte count, platelet count, hematocrit, serum chymase level, aspartate transferase, alanine transferase, serum urea, and serum creatinine.

2.4 | Statistical analysis

All information was collected and collated in Microsoft Excel spreadsheet (MS Office-365). Multivariable logistic regression was utilized for examining the predictive ability of covariates for dengue severity. These include continuous covariates like age, duration of fever at admission, serum chymase level, initial blood counts (like hemoglobin, hematocrit, platelet, total leukocyte count); and categorical covariates like gender, warning signs, hemorrhagic features, and other clinical features. The area under the receiver′s operating characteristics curve (ROC) was calculated for finding accuracy of serum chymase level in predicting severe dengue. We considered area under ROC (AUROCs) to be poor if it is ≤0.7, adequate at 0.7 to 0.8, good at 0.8 to 0.9, and excellent at ≥0.9. Serum chymase level was dichotomized by defining its optimum cut-off using Youden′s-J statistics on the ROC curve. Youden′s J index finds out the cut-off value that maximizes the sum of sensitivity and specificity (or equally minimizes the sum of false positive and false negative errors) and is calculated as J = (sensitivity + specificity − 1).[8,9] Diagnostic statistics of serum chymase level (dichotomized) were calculated for the development of severe dengue. All the above analyses were performed, and graphs were prepared with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. All tests of significance used a two-sided $p \leq .05$. A meta-analysis with adherence to PRISMA guidelines, was performed to summarize the odds ratio (OR) of our result and of previously published studies. Random-effects model was used to weigh the included studies. To assess the heterogeneity among studies, inconsistency statistics (I^2) was used and I^2 greater than 50% were considered for significant heterogeneity. Publication bias was assessed using funnel plots. Meta-analysis was performed with the METAPROP platform for STATA (version-14.2; StataCorp).

3 | RESULTS

3.1 | Patient characteristics

A total of 1129 acute febrile patients were screened for DF, and 338 patients were finally included according to the inclusion criteria (Figure 1). Patients were of mean age of 29.15 years, with two-third consisting of male gender (224 out of 338). At initial presentation, 272 patients (80.5%) were having DF without warning signs and 66 patients (19.5%) were having DF with warning signs. A serum

sample was collected on admission for chymase assay. All the included patients were followed up till four days post-defervescence (i.e., afebrile) and final discharge diagnosis was noted. In due course of illness, 26 out of 338 (7.8%) patients developed severe dengue. Sixty-five patients (19.2%) developed DF with warning signs, and 247 (73%) patients were discharged with a diagnosis of DF without warning signs. Clinical characteristics and laboratory parameters of study subjects are presented in Table 1 and detailed clinical presentation of severe dengue patients are presented in Table S1.

3.2 | Elevated serum chymase, a predictor of dengue severity

Univariate logistic regression analysis showed that clinical features like upper gastrointestinal (UGI) bleed, heavy menstrual bleed, persistent vomiting and abdominal pain; and laboratory parameters like platelet count and serum chymase level at admission were independently associated with a final diagnosis of severe dengue. (Table 2) When adjustment for all covariates was made using multivariate logistic regression analysis, only serum chymase level and initial platelets were able to predict the development of severe dengue (Table 2). The odds of having severe dengue was 1.787 times more likely than the odds of having non-severe dengue (adjusted odds ratio [aOR]: 1.787; 95% CI: 1.309–2.440) with every unit increase in serum chymase level. It was also found that serum chymase was able to predict all the three features of severe dengue, that is, severe plasma leakage (OR: 1.529; 95% CI: 1.187–1.970), severe organ damage (OR: 1.414; 95% CI: 1.104–1.812) and severe bleeding (OR: 1.4; 95% CI: 1.073–1.827). Unlike serum chymase, lower platelet count was associated with severe dengue, with aOR value nearing one (aOR: 0.981; 95% CI: 0.968–0.993).

TABLE 2 Symptoms, serum chymase level, and blood counts associated with the diagnosis of severe dengue versus non-severe dengue (with or without warning signs)

Variables	Unadjusted OR	*p*	Adjusted OR	*p*
Demographic profile				
Age	0.980 (0.943–1.019)	.314	0.966 (0.921–1.015)	.175
Gender	0.567 (0.253–1.269)	.167	0.437 (0.140–1.365)	.154
Fever details				
Day of fever	1.292 (0.687–2.426)	.427	1.050 (0.481–2.293)	.903
Warning signs				
Petechiae/purpura	0.851 (0.108–6.743)	.879	0.069 (0.002–2.148)	.127
UGI bleed	3.595 (1.394–9.269)	.008	1.965 (0.376–10.27)	.423
Nasal bleed	3.261 (0.859–12.38)	.083	1.011 (0.091–11.27)	.993
Hemoptysis	6.200 (0.543–70.76)	.142	1.328 (0.057–31.09)	.860
Gum bleed	3.000 (0.797–11.29)	.104	3.545 (0.535–23.48)	.190
Heavy menses	4.957 (1.231–19.96)	.024	2.758 (0.313–24.30)	.361
Persistent vomiting	3.303 (1.342–8.131)	.009	0.809 (0.078–8.349)	.859
Abdominal pain	2.637 (1.082–6.426)	.033	1.230 (0.131–11.57)	.857
Hepatomegaly	1.650 (0.356–7.642)	.522	1.288 (0.175–9.474)	.803
Third spacing	2.130 (0.584–7.769)	.252	1.177 (0.213–6.507)	.852
Other symptoms				
Diarrhea	2.733 (0.949–7.870)	.062	1.541 (0.397–5.978)	.532
Cough	1.226 (0.348–4.325)	.751	1.849 (0.399–8.566)	.432
Lab values on admission				
Initial TLC	0.925 (0.783–1.092)	.358	0.949 (0.774–1.164)	.916
Initial hemoglobin	0.976 (0.830–1.146)	.763	1.019 (0.712–1.460)	.916
Initial HCT	0.991 (0.931–1.055)	.769	0.986 (0.862–1.127)	.833
Initial platelets	0.982 (0.971–0.994)	.002	0.981 (0.968–0.993)	.003
Serum chymase level	1.587 (1.240–2.030)	.000	1.787 (1.309–2.440)	.000

Note: $p < .05$ is considered significant.

Abbreviations: aOR, adjusted odds ratio; HCT, hematocrit; OR, odds ratio; TLC, total leukocyte count; UGI, upper gastrointestinal.

We next compared the mean serum chymase concentrations of patients grouped on the basis of their final diagnosis. Patients with a final diagnosis of severe dengue had a significantly higher serum chymase levels than all other patients. (Figure 2A). The chymase level was also significantly elevated in the serum of patients with severe dengue, compared with the level in remaining patients, irrespective of the age (Figure 2B) and gender (Figure 2C). In patients presenting on day 3 and 4 of fever, serum chymase levels were significantly elevated in patients having severe dengue, compared with other patients; whereas this was not in the case of patients presenting in earlier course of the disease (day 1 or 2) (Figure 2D). Mean chymase level and their standard deviation according to each grouping characteristics (i.e., age group, gender, and day of fever) is available in Table S2. We also analyzed the serum chymase level according to the older definition of dengue. Patients with a final diagnosis of DSS ($n = 22$; mean chymase: 2.24 ng/ml; *SD*: 1.1), had a significantly higher level of initial serum chymase as compared with DHF ($n = 55$; mean: 1.32 ng/mL; *SD*: 1.5; $p = .011$) and DF ($n = 261$; mean: 1.04; *SD*: 1.1; $p < .001$). But the difference of serum chymase among DHF and DF was not statistically significant ($p = .380$).

AUROC of serum chymase level for predicting severe dengue was 0.835 (95% CI: 0.765–0.905; $p < .001$) (Figure 3), which was significantly higher than the AUROC of platelet count at admission (0.760, 95% CI: 0.650–0.870) ($p < .001$). When evaluated in different age groups, chymase level was an "Excellent" predictor in patients with the age group of 14–18 years (AUROC: 0.916; 95% CI: 0.831–1.000) and "Good" predictor in the age group of 18 to 45 years (AUROC: 0.806, 95% CI: 0.713–0.899). Similarly, chymase level was a "Good" predictor of dengue severity in both the male (AUROC: 0.813; 95% CI: 0.717–0.909) and female (AUROC: 0.859; 95% CI: 0.755–0.962) patients. It was also found to be a "Good" predictor in patients presenting on day 3 (AUROC: 0.822, 95%CI: 0.720–0.923) and day 4 (AUROC: 0.837; 95%CI: 0.729–0.945) of illness. ROC curves, according to each grouping characteristics (i.e., age group, gender, and day of fever) is available in Figure S1–3.

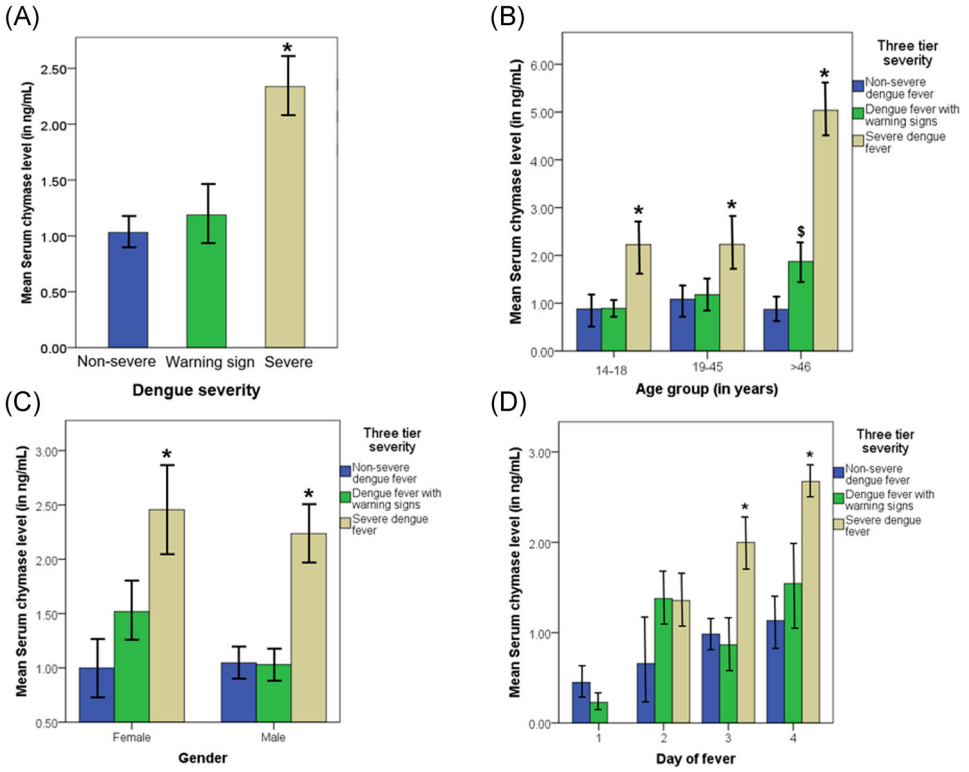

FIGURE 2 Comparison of mean serum chymase level with the final diagnosis. A, Patients with severe dengue had significantly higher levels of serum chymase (mean: 2.34 ng/ml) as compared with patients with dengue with warning signs (mean: 1.19 ng/ml) and without warning signs (mean: 1.04 ng/ml). B, Chymase levels were significantly higher in patients with severe dengue in all age groups than that of patients with dengue with warning signs and without warning signs. C, Chymase levels were significantly elevated in both male and female patients, having severe dengue, compared with levels among patients of dengue with warning signs and without warning signs. D, In patients presenting on day 3 and 4 of fever, serum chymase levels were significantly elevated in severe dengue, compared with levels among patients of dengue with warning signs and without warning signs. *Indicates significantly higher chymase level in severe dengue patients than that of the other two groups, $p < .05$. $ indicates significantly higher chymase level in patients of dengue with warning signs than that of patients not having warning signs, $p < .05$. Error bars indicate 2 standard deviation. Dataset is available in the Table S2

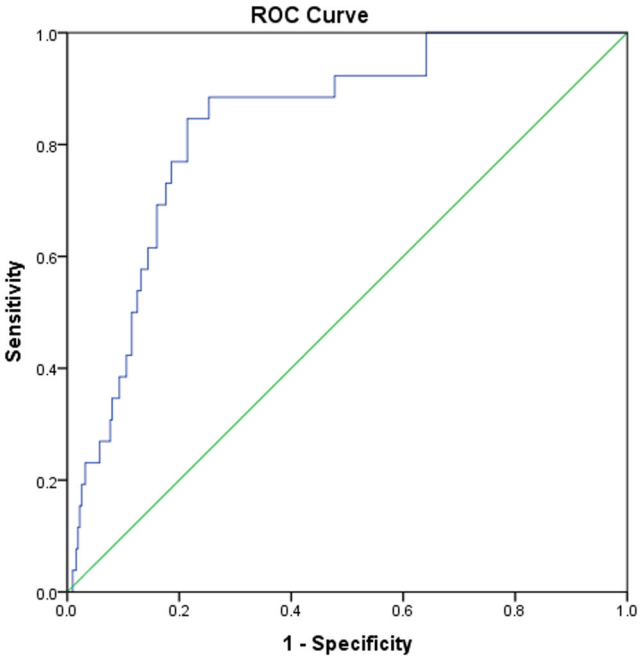

FIGURE 3 Receiver operator characteristic curve (ROC) analysis of chymase level for all patients, with outcome parameter as severe dengue. Area under ROC was 0.835 (95% confidence interval [CI]: 0.765–0.905, $p < .001$), suggesting serum chymase as a "Good" predictor of severe dengue

Serum chymase was also able to predict the different features of severe dengue, that is, severe plasma leakage (AUROC: 0.867; 95% CI: 0.759–0.975), severe bleeding (AUROC: 0.855; 95% CI: 0.803–0.906) and severe organ damage (AUROC: 0.803; 95% CI: 0.701–0.904) (Figure S4–6).

An optimum cut-off value of 1.35 ng/ml of serum chymase concentration was determined for severe dengue. At this level, severe dengue can be predicted with a sensitivity of 88.5%, a specificity of 74.7%, positive predictive value (PPV) of 22.5% and negative predictive value of 98.7%. In addition, serum chymase had a positive likelihood ratio (PLR) of 3.49 and a negative likelihood ratio (NLR) of 0.15.

A meta-analysis of aOR of our study and the study by Tissera et al. resulted in a pooled OR of 1.48 (95% CI: 1.11–1.98, $I^2 = 70.7\%$) (Figure S7). The heterogeneity among the studies could have been aroused from different study population.

4 | DISCUSSION

Our study demonstrated the potential role of serum chymase as an early predictor of dengue severity among non-severe patients with DF. Although few clinical features like UGI bleed, heavy menstrual bleed, persistent vomiting and abdominal pain were associated with the final diagnosis of severe dengue; multivariate analysis showed only serum chymase and platelet count at admission as independent predictors of dengue severity. Further, ROC analysis demonstrated the serum chymase to be an accurate predictor of dengue severity, irrespective of age and gender.

In our study population, the odds of having severe dengue was 1.787 times more likely than the odds of having non-severe dengue with every unit increase in serum chymase level. Tissera et al.[10] had collected data prospectively on 347 dengue patients with fever duration of less than 6 days in Sri Lanka and reported a similar finding that raised initial chymase level in DF patients was indicative of a later diagnosis of DHF or DSS (aOR: 1.32, 95% CI: 1.21–1.44). A similar finding was observed in the meta-analysis of our study and the study by Tissera et al. Furuta et al.[11] conducted a study in Vietnamese children ($n = 103$) aged from 5 to 15 years of age and found the level of chymase were significantly higher in patients with DHF and DSS as compared with those of DF and other febrile controls. Another Vietnamese study by Inokuchi et al.[12] which enrolled adult dengue patients ($n = 111$) and other febrile illness patients as control ($n = 85$), found that plasma chymase concentration was significantly higher in DSS patients as compared with other cases and controls. Similar findings were reported by St John et al.[6] also. Recently, Rathore et al had conducted a similar study in Sri Lanka in 2020 and prospectively recruited 84 pediatric dengue patients (age <12 years). The study reiterated the same conclusion that serum chymase levels are associated with severe dengue disease in hospitalized pediatric patients.[13] This signifies the ability of chymase as an early biomarker for prediction of severe dengue, adjusted for clinical and initial laboratory parameters.[10,14] The range of serum chymase reported in our study was lesser than that of previously published research,[10] which might have aroused due to the use of different reagents for chymase estimation and different study population.

It was found that chymase was more accurate in severity prediction in subgroups of females and adolescents. Tissera et al.[10] and Rathore et al.[13] also demonstrated chymase to be a better predictor among children than that of adults. In our study, patients presenting with a duration of fever from 3 to 4 days, higher chymase was associated with the final diagnosis of severe dengue, whereas the predictive ability for severe dengue was insignificant in patients admitted within two days of fever onset. This may be explained by the fact that only a few patients were included in the study who presented in the early course of their illness. Tissera et al.[10] found a similar predictive ability of chymase in patients with day 3 to 6 of fever, but they did not mention about the subgroup of patients presenting in day 1 or 2 of fever.

In our study, the mean serum chymase was found to be significantly higher in the patients with final diagnosis of severe dengue, as compared with that of DF with or without warning signs (but no difference between patients with or without warning signs). Study by Furuta et al.[11] showed a similar result (i.e., patients with DSS had higher chymase than patients with DSS or DF). But in the studies by Tissera et al.[10] and Rathore et al.,[13] patients with warning signs or DHF had also significantly higher serum chymase

level as compared with DF only. The discrepancy between these studies needs to be investigated further. One of the explanations could be the difference in definitions utilized to classify the dengue severity.

Tissera et al had defined cut-off value of chymase concentration as 1.5 ng/ml and demonstrated it to be 96% sensitive and 79% specific.[10] Similarly, in our study population, chymase with a cut-off value of 1.35 ng/ml (derived from Youden's J analysis of ROC curve) was found to be somewhat less sensitive (88.5%) and specific (74.7%). Even the PPV of chymase was very low, which may be explained by the fact that the proportion of non-severe dengue patients developing severe dengue in our cohort was low.[15] Despite this, PLR (3.49) and NLR (0.19) of serum chymase were acceptable.

In the studies published by Furuta et al.[11] and Inokuchi et al.,[12] measurement of the chymase concentration in sequential serum samples showed that levels decreased over time as acute disease resolved (by fourth day). But in our study, serum samples were collected at the time of admission only, so the trend of serum chymase over time could not be assessed. When we compared the mean chymase level among patients presenting with different duration of fever, there was no significant difference observed.

We have included the patients in the study prospectively. The use of recent WHO-2009 classification of dengue patients made the results of this study appropriate for use in current practice.[3,16,17] But, our study was not free from limitations. It was a single-centered study; hence generalization to a wider population group is not possible. Prevalence of severe dengue on follow up was less, leading to a lower PPV of chymase. The data on serum chymase levels according to the dengur virus (DENV) serotypes was not collected in our study. This might have undermined some important information like specific association of DENV serotypes and mast cell chymase; and the possibility of reinfection (homologous and heterologous) on mast cell activation.

5 | CONCLUSION

The results of this study are to be further validated by robust, multicentric study designs. Controlled trials are warranted to assess whether a higher serum chymase level at admission could promote the allocation of healthcare resources to those who are most likely to develop severe dengue. Nonetheless, this study adds to the scarce literature of serum chymase as an objective predictor of dengue severity.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTINS

Ankit Kumar Sahu: conceptualization, methodology, software, validation, formal analysis, investigation, writing (original draft and editing) and visualisation. Praveen Aggarwal: conceptualization, methodology, formal analysis, investigation, resources, writing (editing), supervision and project administration. Meera Ekka: methodology, writing (editing), supervision. Jamshed Nayer: methodology, writing (editing), formal analysis, writing (editing), supervision and data curation. Sanjeev Bhoi: writing (editing), supervision and project administration. Akshay Kumar: methodology, writing (editing), supervision. Kalpana Luthra: formal analysis, investigation, resources and project administration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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