Original Article

Pro- and anti-inflammatory cytokines signatures at different severity of dengue infection

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Abstract

Context: Dengue disease severity and progression are determined by the host immune response, with both pro- and anti-inflammatory cytokines are key mediators. **Aims:** To study pro- and anti-inflammatory cytokines across dengue severity and as a biomarker for predicting severe dengue infection. **Settings and Design:** Hospital-based cross-sectional study was conducted on 125 dengue-positive subjects across the 5-60 years age group of either gender in 2022. **Methods and Materials:** Haematological parameters and blood samples were drawn to measure cytokines IL6, IL-10 and TNF alpha using the ELISA technique. **Statistical Analysis:** One-way ANOVA and the Kruskal – Wallis test were used to compare the dependent variables across categories of the dengue spectrum. Receiver operating characteristic curve was drawn to calculate the predictability of the cytokines as a predictor of severe dengue. A $P < 0.05$ was considered significant. **Results:** 34.4% of cases had severe dengue infection with 53.2% of severe cases reported in >40 years of age. Only IL-6 levels significantly increased ($P < 0.01$) across the spectrum of dengue infection across age groups >20 years with a consistent and significant fall in platelet levels ($P < 0.01$). The accuracy of IL-6 to predict severe dengue was 74.4% and platelet count was 16.2%. **Conclusions:** Only IL-6 cytokine levels were significantly increased across the spectrum of dengue infection observed in age >20 years and can significantly predict the probability of severe dengue by 74% (sensitivity 81.4%). A significant decrease in platelet values is consistent with the severity but is not a good predictor for severe dengue infection.

Keywords: Cytokines, dengue, DENV, IL-6

Introduction

Dengue fever (DF) is now a ritual epidemic in several parts of India.[1] Dysregulated cytokines[2] and cross-reactive antibody-dependent enhancement trigger a cascade of events, including vascular leakage, coagulopathy, and organ dysfunction, ultimately leading to dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).[3] The intricate balance between pro- and anti-inflammatory cytokines influences viral clearance and dengue-related complications.[4] Poor prognostics in differentiating individuals of DF from those who may progress to DHF and DSS or over-hospitalization poses a challenge in clinical management and detection of outbreaks.[5] Using cytokine profile patterns as biomarkers will help for therapeutic interventions and improve patient outcomes;[6] hence, this study investigates levels of pro- and anti-inflammatory cytokines across the spectrum of dengue infection and their association with severe dengue infection.

Subjects and Methods

A cross-sectional study was initiated to observe the levels of the TNFα, IL 10, and IL 6, cytokines, and their association with the spectrum of dengue virus infection in the diagnosed cases of

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patients with dengue during the outbreak of dengue in the year 2022 in Dehradun and Rishikesh. The study was initiated under the Department of Physiology and Microbiology, Swami Rama Himalayan University, Dehradun over 12 months after obtaining ethical clearance from the university. The study included blood samples of diagnosed cases of dengue across the 5-60 years age group of either gender attended OPD or admitted indoors in the hospital at HIMS Dehradun and Rishikesh, after obtaining written informed consent from them and their parents/guardian in case of minor for their inclusion in the study.

Sample size

With the hospital-based prevalence of dengue confirmed cases during an epidemic of 51.2% of the Indian population[7] and using a power of 80% at a 5% level of significance, a sample size of 100 cases of suspected dengue was arrived at considering a 10% absolute error in the prevalence [sample size $4pq/d^2$; where "p" is prevalence, "q" is 1-p, and "d" is an absolute error]. Considering 10% of attrition due to lost to follow-up, aberrant measurements, and wastage in tests and/or increasing the power of the analysis, the sample is increased to a minimum of 125. Fifty healthy controls of a similar age group were enrolled for cytokines measurements to get a baseline for comparison.

Selection of subjects

The sample was collected from HIMS hospital and Rishikesh government hospital from those who were confirmed cases of dengue infection and tested positive for NS1 and immunoglobin tests (Igs] for dengue.[8] Inclusion criteria included all dengue cases: clinical symptoms and confirmed cases of dengue with positive NS1 and raised immunoglobin IgM/IgG for dengue[8] of age groups (5–60 years) of either gender. Healthy controls of similar age groups with no clinical symptoms suggestive of any infection were also measured for levels of cytokines to help identify the baseline of cytokines for comparison. Exclusion criteria included cases of non-dengue infection, such as acute respiratory diseases, pneumonia, sinusitis, or UTI. Dengue-positive cases by NS1 and Ig [s] cases were grouped for analysis according to WHO's revised classification 2009 based on the index of severity, such as plasma leak, bleeding, and organ involvement.[8]

Study protocol

Following informed written consent, the demographic parameters, clinical features, and medical and addiction history were recorded from the included cases of dengue infection. Investigatory reports of haematological tests, including haemoglobin, total leucocyte counts, and platelets, were recorded from the reports of the cases from bedside reports from the IPD cases and on revisit from OPD cases. Five millilitres of blood was drawn, which was used to measure cytokines levels of IL6, IL10, and TNF using the ELISA technique using the Dichlone kit abiding by the protocol as per the kit. Blood of the 50 healthy controls of a similar age group was also investigated for the same cytokines levels to identify the baseline.

Data management and statistical analysis

Data were analysed using the statistical program SPSS for Windows, version 20 (SPSS Inc., Chicago, IL, USA). The study case was grouped as per the severity of dengue based on the WHO classification 2009[8] and also compared across age groups. The analysis was based on the Shapiro − Wilks test for normality of the data. Results are tabled as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) for normal data and Kruskal–Wallis tests for non-normal data were used to determine differences in haematological and cytokines levels between different spectra of dengue infection. The Mann–Whitney *U*-test was used for comparisons of non-parametric data between two groups. Receiver operating characteristic (ROC) curve analysis was performed to determine how well they may predict cases of severe dengue fever. Accuracy was determined using the Youden index, which is calculated as (sensitivity and 1-specificity). Statistical significance was defined as a $P < 0.05$.

Results

The study was conducted on 125 dengue-diagnosed cases during the year 2022-2023 reported at the Himalayan Hospital and Government Hospital at Rishikesh, and the following observations of the demographic and clinical symptoms were analysed. For analysis, the dengue patients were classified as mild (dengue fever without warning sign), moderate (dengue fever with a warning sign) and severe based on the WHO criteria 2009.[8]

Table 1 shows that most cases were mild (dengue fever) and moderate (dengue fever with warning signs) in nature presented in the age less than 40 years. Most of the severe cases reported were from the age more than 40 years (53.5%). Male predominance across all spectra of the dengue infection was observed to be >50%, but statistically, no difference in dengue presentation was observed between males and females. Severe cases reported a positive history of addictions and co-morbidities as compared to less than 40 years. All the patients had a history of fever with 95% of cases having myalgia. Rashes were present in those with mild and severe dengue infection in ~ 40% of cases.

Table 2 shows the haematological and immunological parameters across the spectrum of severity of dengue. The average haemoglobin was within the normal range in all the spectrum of dengue infection with a marginal fall in severe cases (12.40 ± 2.66 mg/dl) in severe dengue infection, but the difference was statistically non-significant ($P = 0.68$). Platelet counts decreased significantly from 1.2 lakhs in mild dengue to 70,000 per cumm in severe cases, which was expected based on the severity of dengue ($P \leq 0.001$). Mild and moderate dengue cases had a lower total leucocyte count as compared to severe cases of 8.06 ± 11.7 thousand/ml, and the fall was statistically significant ($P = 0.03$). Although the values of total leucocyte count were higher in severe cases, it was within normal limits of health.

Immunological marker of inflammation IL-6 was observed to be significantly raised from mild to severe cases and was statistically significant ($P \leq 0.001$). No significant difference in the levels of pro-inflammatory cytokine TNF was observed although its level increased from mild to severe cases ($P = 0.41$). Anti-inflammatory marker IL-10 also did not vary significantly from mild to severe although their levels increased.

Table 3 shows that on observing the haematological and immunological parameters across different age groups in the spectrum of dengue infection, the mean levels of platelet decreased in all the age groups with the significant decrease in the age 20 years and beyond ($P \leq 0.001$ and 0.01). The pro- and anti-inflammatory cytokine levels were also raised with the severity of dengue infection across all the age groups, but only IL-6 mean values were raised significantly in the age group of 20-40 years ($P < 0.001$) and > 40 years ($P = 0.012$). The TNF-alpha mean values increased from mild to severe infection across all age groups, but the rise was statistically insignificant.

Analysing the interaction of the age group and the severity of the dengue infection on the dependent variable IL-6 and platelets by two-way ANOVA, it was observed that the spectrum alone contributed 15 times of effect on the measurements of the cytokines and the contribution is significant ($P < 0.001$). Figure 1 shows that the interaction of the age group and spectrum of disease contributed by only 3.3 times effect on higher cytokines.

Analysing the interaction of the age group and the severity of the dengue infection on the dependent variable platelets, it was observed that the spectrum alone had 25 times effect on the measurements of lower values of platelets and was significant ($P < 0.001$). Figure 2 shows that the interaction of the age group and spectrum of disease contributed a 4.9 times effect on lower platelet counts.

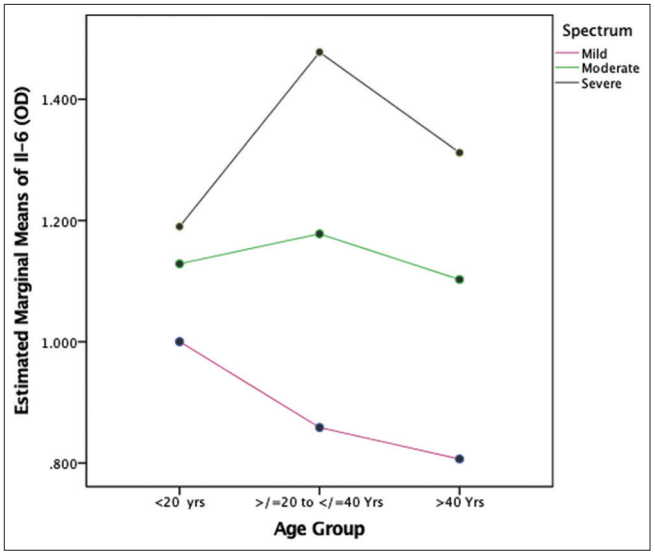

Figure 1: Mean Values of the IL-6 (OD) in Spectrum of Dengue Infection Across Age Groups (n=125)

Table 1: Demographic, medical history and dengue symptoms among spectrum of dengue positive cases (*n*=125)

Parameter	Mild (*n*=54) Dengue Fever	Moderate (*n*=28) Dengue Fever Without Warning Sign	Severe (*n*=43)
Age (years)			
0−20	24 (44.4%)	12 (42.9%)	5 (11.6%)
20−40	25 (46.3%)	9 (32.1%)	15 (34.9%)
40−60	5 (9.3%)	7 (25%)	23 (53.5%)
Gender			
Male	28 (51.9%)	15 (53.6%)	24 (55.8%)
Female	26 (48.1%)	13 (46.4%)	19 (44.2%)
Medical history			
Addiction (alcohol, tobacco, smoking, drug abuse)	7	1	11
H/O chronic diseases (HT, DM, TB, COPD)	1	0	6
Symptoms of dengue			
Fever	54 (100%)	28 (100%)	34 (100%)
Myalgia	51 (94.4%)	27 (96.4%)	41 (95.3%)
Rashes	24 (44.4%)	1 (3.6%)	16 (37.2%)

Table 2: Haematological and immunological parameters across severity of dengue infection (*n*=125)

Parameter	Mild Dengue Fever (Mean±SD)	Moderate Dengue Fever Without Warning Sign (Mean±SD)	Severe (Mean±SD)	Kruskal−Wallis, *P*
Haematological				
Haemoglobin (g/dl)	12.99±1.95	13.28±2.11	12.40±2.66	0.68
Platelets (Lac/cumm)	1.33±0.59##	0.93±0.75$$	0.709±0.51**	0.000
Total Leucocyte count (Thousand/cumm)	4.65±2.41	4.68±2.84$$	8.06±11.7*	0.037
Immunological				
IL-6 (OD)	0.94±0.38##	1.13±0.32	1.29±0.51**	0.000
IL-10 (OD)	0.93±0.43	1.07±0.51	1.16±0.59	0.23
TNα (OD)	0.96±0.46	1.01±0.44	1.13±0.63	0.41

$$Between moderate and severe; ##between mild and moderate; **between mild and severe

Table 3: Age group wise haematological and immunological parameters across severity of dengue infection (*n*=125)

Parameter	Mild Dengue Fever (Mean±SD)	Moderate Dengue Fever without Warning Sign (Mean±SD)	Severe (Mean±SD)	Kruskal–Wallis, *P*
Age group in years				
Age <20				
Haematological				
Haemoglobin (g/dl)	12.47±2.02	12.92±2.09	11.8±2.77	0.63
Platelets (Lac/cumm)	1.37±0.67	1.16±0.31	0.69±0.40	0.05
Total leucocyte count (Thousand/cumm)	4.68±2.55	5.69±3.84	6.53±2.54	0.25
Immunological				
IL-6 (OD)	0.99±0.36	1.12±0.37	1.19±0.45	0.45
IL-10 (OD)	1.03±0.46	1.11±0.37	1.20±0.59	0.72
TNα (OD)	1.15±0.43	1.18±0.48	1.42±1.04	0.59
Age, 20–40				
Haematological				
Haemoglobin (g/dl)	13.54±1.70	14.31±2.22	13.50±2.33	0.58
Platelets (Lac/cumm)	1.24±0.42	0.65±0.28	0.50±0.29	0.00**
Total leucocyte count (Thousand/cumm)	4.68±2.35	3.87±1.71	9.88±18.9	0.32
Immunological				
IL-6 (OD)	0.85±0.35	1.17±0.31	1.47±0.46	0.00**
IL-10 (OD)	0.86±0.42	1.05±0.60	1.15±0.62	0.38
TNα (OD)	0.75±0.32	0.88±0.26	1.03±0.62	0.24
Age, 41–60				
Haematological				
Haemoglobin (g/dl)	12.62±2.40	12.59±1.74	11.78±2.73	0.66
Platelets (Lac/cumm)	1.62±0.87	0.72±0.28	0.62±0.27	0.01**
Total leucocyte count (Thousand/cumm)	4.41±2.54	3.99±1.33	7.21±5.37	0.18
Immunological				
IL-6 (OD)	0.80±0.14	1.10±0.46	1.31±0.41	0.012*
IL-10 (OD)	0.81±0.15	1.01±0.66	1.15±0.59	0.66
TNα (OD)	0.71±0.37	1.02±0.43	1.13±0.54	0.24

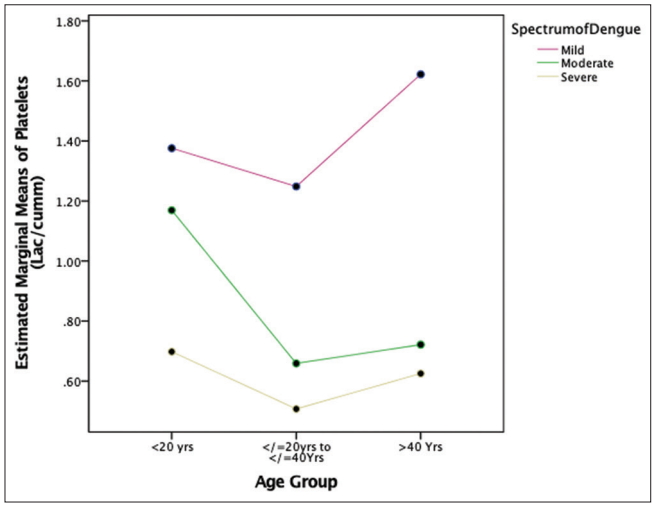

Figure 2: Mean Values of the Platelets (Lac/cumm) In Spectrum of Dengue Infection Across Age groups (n=125)

To determine the potential/predictive haematological and cytokines biomarkers of dengue severity, ROC curves were drawn for mild, moderate and severe dengue infection. The ROC curve was plotted only for platelets and IL6 cytokine and showed a significant difference in statistical analysis for predicting severe dengue and differentiating it from dengue without warning sign symptoms and dengue with warning signs. AUC plots depict the trade-off between sensitivity and 1-specificity. The closer the curve follows the left-hand border and the top border of the ROC space, the more accurate the test [Figure 3a]. ROC curve compared decreased platelets between mild (dengue fever), moderate (dengue with warning sign) symptoms and severe dengue [Figure 3b]. ROC curve compared increased IL-6 levels between mild (dengue fever), moderate (dengue with warning sign) and severe dengue.

Figure 3a and 3b shows the ROC curve for the predictive value of IL6 and platelet counts for the development of severe dengue in dengue-positive cases across all age groups. The accuracy of these to predict severe dengue was 74.4% ($P \leq 0.001$) for IL-6 and 16.2% ($P \leq 0.001$) for platelet counts both of which were found to be statistically significant. The cut-off values of IL-6 and platelet counts were 0.909 OD and 0.905 lakh/cumm for predicting severe dengue with the sensitivity of 81.4% and specificity of 51.2% and sensitivity of 74.1% and specificity of 70.4%, respectively [Table 4].

Discussion

Dengue virus inoculation in the dermis primarily infects the tissue macrophage system releasing pro-inflammatory cytokines, such as the interferons and tumour necrosis factor (TNF), which are responsible for clinical features, such as fever, rashes, and myalgia, during the primary infection.[9] Moreover, antibodies to NS1 antigen once formed cross-react with endothelial cells

Table 4: Predictive value of IL6 and platelets counts for development of severe dengue in dengue positive cases across 5–60 years age groups

S No.	Test Result Variable	Area	Std Error	Significance Level	95% Confidence Interval	
					Lower Bound	Upper Bound
Figure 3a	Platelet (Lac/cumm)	0.16	0.035	0.001	0.093	0.231
Figure 3b	IL-6 (OD)	0.744	0.046	0.001	0.655	0.834

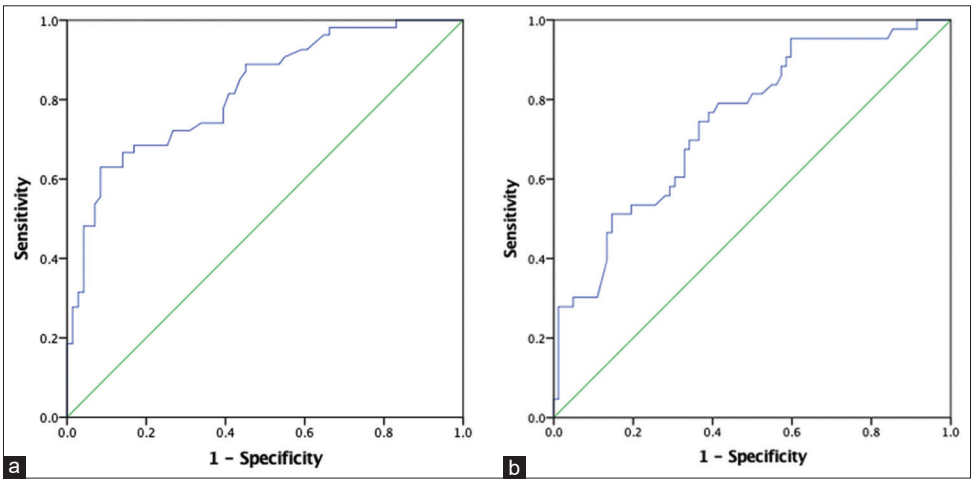

Figure 3: (a) AUC For Relation of the Platelets (Lac/cumm) Between Spectrum of Dengue Infection(n=125), (b) AUC For Relation of the IL-6 (OD) Between Spectrum of Dengue Infection (n=125)

and could trigger these cells to express nitric oxide (NO) and undergo apoptosis.[10] During progression to severe form, more infected cells die due to apoptosis and necrosis, releasing the virions, cytokines and toxic products causing a decrease in the platelets development by stromal cells, activation of the coagulation and platelet dysfunction by possible tropism for endothelial cell, leading to petechiae increased vascularity and easy bruising,[11] which is a characteristic of the DHF, especially during cross-reactivity of immunoglobins in secondary dengue. Overstimulation of cross-reactive T cells during secondary infection of the heterologous virus could exacerbate rather than mitigate disease by delaying virus clearance, while producing high levels of proinflammatory cytokines and other mediators by the abortively infected bystanders leading to more organ damage, especially liver, more vascular leak and coagulopathy characteristics of DSS by a phenomenon that is claimed to be caused by antibodies and termed antibody-dependent enhancement (ADE) of disease.[12] The observation that DHF/DSS is primarily found in a small percentage of secondary infections even in virulent strains and in a much smaller amount in primary infections shows the importance of host determinants in cytokines effect and progression to severe disease.

Protective pro-inflammatory cytokines, such as interferon-gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6) and IL-10, are produced by dengue virus-infected cells to draw and activate leucocytes at the site of infection.[13] IL-6 together with other pro-inflammatory cytokines potentiates the coagulation cascade and downregulates the production of TNF and TNF receptors and is a potent inducer of fever. TNF enhances capillary permeability and activates the fibrinolysis system. IL-10 produced by monocytes and regulatory T helper cells may cause platelet decay and downregulate the inflammatory response creating a proviral survival milieu. As the relationship between cytokines activation in response to the virus is complex, it is difficult to gauge the severity of infection. Previous reports have identified the role of IL-6, TNF α and IL-10 in the progression of disease; however, the association of these with the severity is limited.[14] Our study investigated the levels of these cytokines and their association in patients with severe dengue infection and identified biomarkers of increased disease progression in dengue infection.

34.4% of the dengue cases were of the severe category. Our study observed that 53% of severe cases were >40 years of age, and <40 years usually had only mild to moderate form of dengue during this regional epidemic of 2022. Male predominance was observed but was not significantly higher than females. No difference in dengue incidence in males and females could be because of the regional proportion of the gender distribution in Uttarakhand with women contributing to earning by doing work with equal exposure away from home. Similar reports were observed from the 2007 epidemic of Haldwani city outbreak[15] and the 2012-2013 epidemic report of Dehradun.[16] Several studies in north and western India observed male predominance and significant association ($P < 0.001$) with dengue in paediatric population[17] and in both rural and semiurban areas.[18] A contrasting large epidemiological serological study of dengue in India among 700 patients suspected of having dengue has reported a 40% seropositivity and haemorrhagic findings with the greater propensity in females with significant association with dengue[19] but the small sample size in the study did not reflect the

actual gender-based prevalence of the infected population. No significant association of gender with the severity of dengue was observed in several studies,[20,21] which was similar to the finding in our study. Contrarily, few studies have revealed a substantial link between gender and dengue severity in women,[22] while others have suggested that men are more likely to acquire severe dengue.[23]

In our study of the 2021-2022 dengue epidemic, the disease was proportionally more in 20-40 years [39.4%] with 34.9% of them with severe dengue followed by 31.8% in age <20 years. There is a shift in the high proportion of dengue cases in 21-30 years age (695/2354) in the 2010 epidemic at Nainital and Dehradun[15] to epidemic of 2012-2013 in Dehradun (47.85% of cases in age group of 20-40 years followed by 28.6% cases in >40 years of age and 23.6% of case between 12 and 20 years.[16] A study across North Indian Tertiary care hospitals also observed 20-30 years being the most affected group during the 2012-2017 epidemic followed by cases in the lower age <20 years and >40 year of age group.[7] When dichotomized into age groups of 40 years and >40 years, the study by Agrawal *et al*.[20] indicated no significant connection between age and severe dengue, and these findings were supported by others.[21] However, only a few studies have found a strong correlation between severe dengue and the young age group[24] and with the older age group.[23]

Our study observed no difference in the haemoglobin levels across the spectrum of dengue infection with near normal levels (12.4 ± 2.66 g/dl). The platelet count decreased significantly from mild to severe dengue as it has always been a criterion used by WHO for the classification of dengue clinical severity with a count less than 150,000 per microliter of blood.[8] Our study also observed a significant relationship of thrombocytopenia with >40 years and severe dengue because of the suppression of the bone marrow progenitor cells' function[25] and peripheral destruction of platelets.[26] Similar to our study, a rural study in southern India also observed a significant fall in the platelet counts and its association across the different age groups and across the severity of dengue with a larger fall in the age group of <15 years and more in severe form of dengue.[27]

While the activation of immune responses, particularly cytokines, is necessary to control viral replication, too many cytokines lead to an increase in inflammation and the development of illness. Elevated profiles of pro-inflammatory cytokines have been demonstrated in patients with dengue fever, dengue without warning signs, or dengue with warning signs; however, information on severe dengue is limited. In our study, biomarkers of inflammation TNF alpha and IL-6 were raised with the severity of the disease but the rise in IL-6 levels was significant ($P \leq 0.001$). Anti-inflammatory IL-10 cytokine levels did increase from mild to severe cases of dengue but were insignificant. Observation of the increase in IL-6 levels in our study is in concurrence with that observed in dengue haemorrhagic fever patients[28] and is consistent with most studies.[29,30] The rise in the IL-6 levels in dengue patients is variable with some studies showing a rise in IL-6 levels and its association with dengue disease severity[31] while others reporting lower levels of IL-6 in patients with shock,[32] and in some cases, the significant elevation was only observed between dengue fever and dengue haemorrhagic fever. An association of IL6 with thrombocytopenia (<1 lac) was also observed which reflects the severity of the disease.[33] Several studies have observed a rise in both IL6 and TNFα and their association with severe dengue infection[33,34] with some showing insignificant rise in IL-16 in severe dengue vs nonsevere dengue.[35] A rising IL-6 level seen in our study is also reflected by a relative increase in the total leucocyte observed in severe dengue cases which concurrence with IL-6-induced leukocyte recruitment, local inflammation, and damage to endothelial cells related to outcomes like DHF/DSS.[32]

Our study observed a rise in the levels of IL-10 across the spectrum of dengue infection but the rise was insignificant and is in concurrence with a study in Taiwan, which observed significantly increased IL-6 levels ($P < 0.005$) but an insignificant increase in IL-10 levels across the spectrum of dengue infection[36] and contrast to western India during 2017 that observed a significant increase in the cytokines IL10 but in patients with secondary DENV infection as compared to primary.[37] In contrast to our finding, serum IL-10 levels were significantly higher ($P = 0.001$) in patients with severe dengue, when compared to non-severe dengue but does not have a good predictor discriminating those who were likely to develop severe dengue (AUC = 0.66),[38] while some proved IL-10 to be the largest predictor of dengue infection and discriminating DF and DHF with cut off values of IL-10 >134 pg/ml.[28]

TNF (tumour necrosis factor) has been reported to be associated with severity in DHF and is raised in dengue with warning signs[39] but its role in severe dengue is still not clear. The observation of raised TNFα in dengue as compared with healthy controls is in line with other studies.[40] Our study observed an increase in the levels of TNFα from mild to severe dengue cases across all age groups though the rise was non-significant. The results are similar to the study on cytokines in northern India[35] where the difference was statistically significant in contrary to our finding, although the severity was grouped as non-severe and severe and was associated with severity in contrast to our study. Higher level of TNF-α cytokine was associated with severe dengue infection in various other studies.[33,34] There is variability in the levels of TNF alpha across patterns of dengue infection with some studies not observing any statistical difference in the levels of TNF-α in DHF patients compared to DSS and DF patients, but observed the positive and significant association of TNF alpha only in DHF and DSS form of dengue not in the mild DF group.[41] Nevertheless, several investigations have not found a substantial increase in the TNF level in cases of severe dengue.[33,42] The changes in viral serotype, sampling period, clinical presentation of patients, and host TNF mutations are likely to be responsible for the variances in TNF levels between studies.

In agreement, the simultaneous increase in IL-6 and IL-10 in DHF[32] and TNF alpha across the spectrum of dengue infection

is indicative of the dysregulated immune response that leads to the progression to dengue severity. With wide range of cytokines seen in patients with severe dengue and milder forms of dengue may likely be due to the complex nature of this disease and serial testing of the levels can predict with precise time the patient enters the critical severe phase of dengue.

Conclusion

The study concluded that both pro- and anti-inflammatory cytokines are raised with an increase in the severity of infection across all age groups. Increased in IL-6 cytokine levels and decrease in platelet levels are significantly related to an increase in the severity of dengue infection and show a positive relation to the severity in >20 years of age group. Although IL6 increase is a good sensitive indicator [sensitivity of 81.4%] but cannot be concluded as a good bio predictor for severe dengue (probability of severe dengue by 74%). The decrease in platelet values is consistent and statistically significant with the severity as expected clinically but is not a good predictor for severe dengue infection in dengue fever.

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Conflicts of interest

There are no conflicts of interest.

References

1. National Centre for Vector Borne Diseases Control Dengue/DHF situation in India: National Centre for Vector Borne Diseases Control. Available from: https://ncvbdc.mohfw.gov.in/index4.php?lang=1&level=0&linkid=431&lid=3715. [Last accessed on 2023 Jan 24].
2. Katzelnick LC, Gresh L, Halloran ME, Mercado JC, Kuan G, Gordon A, *et al.* Antibody-dependent enhancement of severe dengue disease in humans. Science 2017;358:929-32.
3. Aguilar-Briseno JA, Moser J, Rodenhuis-Zybert IA. Understanding immunopathology of severe dengue: Lessons learnt from sepsis. Curr Opin Virol 2020;43:41-9.
4. Couper KN, Blount DG, Riley EM. IL-10: The master regulator of immunity to infection. J Immunol 2008;180:5771-7.
5. Thein TL, Gan VC, Lye DC, Yung CF, Leo YS. Utilities and limitations of the World Health Organization 2009 warning signs for adult dengue severity. PLoS Negl Trop Dis 2013;7:e2023. doi: 10.1371/journal.pntd.0002023.
6. Bhatt P, Sabeena SP, Varma M, Arunkumar G. Current understanding of the pathogenesis of dengue virus infection. Curr Microbiol 2021;78:17-32.
7. Dinkar A, Singh J. Dengue infection in North India: An experience of a tertiary care center from 2012 to 2017. Tzu Chi Medical Journal 2020;32:36-40.
8. World Health Organization. (2009). Dengue guidelines for diagnosis, treatment, prevention and control : new edition. World Health Organization. https://iris.who.int/handle/10665/44188
9. Durbin AP, Vargas MJ, Wanionek K, Hammond SN, Gordon A, Rocha C, *et al.* Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. Virology 2008;376:429-35.
10. Lin CF, Lei HY, Shiau AL, Liu HS, Yeh TM, Chen SH, *et al.* Endothelial cell apoptosis induced by antibodies against dengue virus nonstructural protein 1 via production of nitric oxide. J Immunol 2002;169:657-64.
11. Nakao S, Lai CJ, Young NS. Dengue virus, a flavivirus, propagates in human bone marrow progenitors and hematopoietic cell lines. Blood 1989;74:1235-40.
12. Halstead SB. Observations related to pathogensis of dengue hemorrhagic fever. VI. Hypotheses and discussion. Yale J Biol Med 1970;42:350-62.
13. Castillo-Macías A, Salinas-Carmona MC, Torres-López E. Immunology of viral infections with a high impact in Mexico: Dengue, Chikungunya, and Zika. Med Univ 2017;19:198-207.
14. Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: An integrated view. Clin Microbiol Rev 2009;22:564-81.
15. Sarkar M, Kumar K, Sharma AK, Gupta AK. Ento-epidemiological characterization of Dengue in Uttarakhand (India). Journal of Mosquito Research 2015 Oct 29;5.
16. Singh R, Singh SP, Ahmad N. A study of clinical and laboratory profile of dengue fever in a tertiary care centre of Uttarakhand, India. Int J Res Med Sci 2014;2:160-3.
17. Badoni G, Gupta P, Pai MO, Kaistha N, Ratho R, Sokeechand N. Dengue burden and circulation of dengue-2 serotype among children along with clinical profiling in Uttarakhand, India: A cross-sectional study from 2018 to 2020. Cureus 2023;15:e33913. doi: 10.7759/cureus.33913.
18. Kumar M, Verma RK, Mishra B. Prevalence of dengue fever in Western Uttar Pradesh, India: A gender-based study. Int J Appl Basic Med Res 2020;10:8-11.
19. Chakravarti A, Arora R, Luxemburger C. Fifty years of dengue in India. Trans R Soc Trop Med Hyg 2012;106:273-82.
20. Agrawal VK, Prusty BSK, Reddy CS, Mohan Reddy GK, Agrawal RK, Sekher Srinivasarao Bandaru VC. Clinical profile and predictors of severe dengue disease: A study from South India. Caspian J Intern Med 2018;9:334-40.
21. Chatterjee N, Mukhopadhyay M, Ghosh S, Mondol M, Das C, Patar K. An observational study of dengue fever in a tertiary care hospital of eastern India. J Assoc Physicians India 2014;62:224-7.
22. Carrasco LR, Leo YS, Cook AR, Lee VJ, Thein TL, Go CJ, *et al.* Predictive tools for severe dengue conforming to World Health Organization 2009 criteria. PLoS Negl Trop Dis 2014;8:e2972. doi: 10.1371/journal.pntd.0002972.
23. Thomas L, Brouste Y, Najioullah F, Hochedez P, Hatchuel Y, Moravie V, *et al.* Predictors of severe manifestations in a cohort of adult dengue patients. J Clin Virol 2010;48:96-9.
24. Mena Lora AJ, Fernandez J, Morales A, Soto Y, Feris-Iglesias J, Brito MO. Disease severity and mortality caused by dengue in a Dominican pediatric population. Am J Trop Med Hyg 2014;90:169-72.
25. Murgue B, Cassar O, Guigon M, Chungue E. Dengue virus inhibits human hematopoietic progenitor growth *in vitro*. J Infect Dis 1997;175:1497-501.
26. Lin CF, Lei HY, Liu CC, Liu HS, Yeh TM, Wang ST, *et al.* Generation of IgM anti-platelet autoantibody in dengue patients. J Med Virol 2001;63:143-9.

27. Khan DM, KuppuSamy K, Sumathi S, Mrinalini VR. Evaluation of thrombocytopenia in dengue infection along with seasonal variation in rural Melmaruvathur. J Clin Diagn Res 2014;8:39-42.
28. Flores-Mendoza LK, Estrada-Jiménez T, Sedeño-Monge V, Moreno M, Manjarrez MD, González-Ochoa G, *et al.* IL-10 and socs3 are predictive biomarkers of dengue hemorrhagic fever. Mediators of inflammation 2017 Oct;2017.
29. Malavige GN, Huang LC, Salimi M, Gomes L, Jayaratne SD, Ogg GS. Cellular and cytokine correlates of severe dengue infection. PLoS One 2012;7:e50387.
30. Restrepo BN, Isaza DM, Salazar CL, Ramírez R, Ospina M, Alvarez LG. Serum levels of interleukin-6, tumor necrosis factor-alpha and interferon-gama in infants with and without dengue. Rev Soc Bras Med Trop 2008;41:6-10.
31. Butthep P, Chunhakan S, Yoksan S, Tangnararatchakit K, Chuansumrit A. Alteration of cytokines and chemokines during febrile episodes associated with endothelial cell damage and plasma leakage in dengue hemorrhagic fever. Pediatr Infect Dis J 2012;31:e232-8.
32. Bethell DB, Flobbe K, Xuan C, Phuong T, Day NP, Phuong PT, *et al.* Pathophysiologic and prognostic role of cytokines in dengue hemorrhagic fever. J Infect Dis 1998;177:778-82.
33. Priyadarshini D, Gadia RR, Tripathy A, Gurukumar KR, Bhagat A, Patwardhan S, *et al.* Clinical findings and pro-inflammatory cytokines in dengue patients in Western India: A facility-based study. PloS One 2010;5:e8709.
34. Bozza FA, Cruz OG, Zagne SM, Azeredo EL, Nogueira RM, Assis EF, *et al.* Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. BMC infectious diseases 2008;8:1-1.
35. Sehrawat P, Biswas A, Kumar P, Singla P, Wig N, Dar L, *et al.* Role of cytokines as molecular marker of dengue severity. Mediterr J Hematol Infect Dis 2018;10(1). doi: 10.4084/MJHID.2018.023.
36. Puc I, Ho TC, Yen KL, Vats A, Tsai JJ, Chen PL, *et al.* Cytokine signature of dengue patients at different severity of the disease. Int J Mol Sci 2021;22:2879.
37. Meena AA, Murugesan A, Sopnajothi S, Yong YK, Ganesh PS, Vimali IJ, *et al.* Increase of plasma TNF-α is associated with decreased levels of blood platelets in clinical dengue infection. Viral Immunol 2020;33:54-60.
38. Malavige GN, Gomes L, Alles L, Chang T, Salimi M, Fernando S, *et al.* Serum IL-10 as a marker of severe dengue infection. BMC Infect Dis 2013;13:1-7.
39. Vitarana T, de Silva H, Withana N, Gunasekera C. Elevated tumour necrosis factor in dengue fever and dengue haemorrhagic fever. Ceylon Med J 1991;36:63-5.
40. Masood KI, Jamil B, Rahim M, Islam M, Farhan M, Hasan Z. Role of TNF α, IL-6 and CXCL10 in dengue disease severity. Iran J Microbiol 2018;10:202-7.
41. Soundravally R, Hoti SL, Patil SA, Cleetus CC, Zachariah B, Kadhiravan T, *et al.* Association between proinflammatory cytokines and lipid peroxidation in patients with severe dengue disease around defervescence. Int J Infect Dis 2014;18:68-72.
42. Rathakrishnan A, Wang SM, Hu Y, Khan AM, Ponnampalavanar S, Lum LC, *et al.* Cytokine expression profile of dengue patients at different phases of illness. PloS One 2012;7:e52215.