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Original Article

Role of Cytokines as Molecular Marker of Dengue Severity

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Abstract. ***Objective:*** **Dengue infection is a rapidly spreading vector-borne disease and is endemic in the Indian subcontinent. It has varied manifestations ranging from subclinical infection to severe fatal shock syndrome. This study aimed to estimate cytokine level in dengue patients and correlate them with dengue severity.**
Methods: **Cases of dengue fever diagnosed in the department of medicine of our institute from July 2015 to November 2016 were included in the study. The clinical features, biochemical, hematological and radiological parameters along with cytokine levels (Interferon-gamma, Interleukin-6, and Tumour Necrosis Factor-alpha) were recorded in all patients.**
Results: **Out of 80 confirmed cases of dengue included in the study, 50 had nonsevere dengue (Group 1), and 30 patients had severe dengue (Group 2). The median level of serum TNF-α in group 2 (62.5 pg/mL) was significantly higher than the median level in group 1 (20 pg/mL), (*p*=0.043). Similarly, the median level of serum IFN-γ in group 2 (10.25 pg/mL) was significantly higher than the median level in group 1 (8.5 pg/mL), (*p*=0.002). The median level of IL-6 was also higher in group 2 (29 pg/ml) as compared group 1(14.2 pg/ml), but this result was not significant (p>0.05).**
Conclusion: **Some cytokines may play a role in the pathogenesis of severe manifestations of dengue.**

Keywords: Dengue infection, Cytokine storm, TNF-α, IFN-γ.

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Introduction. Dengue infection is a common vector-borne disease which can cause a myriad of features ranging from mild febrile episode to severe manifestations like catastrophic bleeding and organ impairment. South East Asia Region (SEAR) and western Pacific region bear nearly 75% of the current global disease burden due to dengue.[1] The pathological basis of dengue fever lies in a complex series of immunological response resulting in a rapid increase in the levels of cytokine and other chemical mediators that are central to the severe manifestations of dengue hemorrhagic fever, such as plasma leakage, shock, and bleeding.[2] However, at the onset of illness, it is difficult to predict which dengue patients are going to progress to severe dengue. By identifying the predictors of severity of dengue, we can target those patients who are likely to proceed to severe dengue, thereby reducing the morbidity and mortality related to dengue infection.

Material and Methods. This was a cross-sectional comparative study conducted in the department of medicine at All India Institute of Medical Sciences, New Delhi between July 2015 and November 2016; it was approved by the ethical committee of the institute. During this period, 102 cases of suspected dengue fever who attended our outpatient and emergency department were screened for dengue fever. Six patients were excluded during screening (three patients tested positive for malaria, one patient had tuberculosis, and two had chronic kidney disease). Samples from remaining 96 patients were subjected to a confirmatory test for dengue, and 80 patients were confirmed positive. Patients presenting within five days of onset of fever were tested for NS1 antigen in serum and those after the fifth day were tested for IgM antibody in serum. Both tests were done using enzyme-linked immunosorbent assay (ELISA) based kits. The patients suffering from other acute febrile illness and chronic diseases like tuberculosis, HIV, and hepatitis were excluded from the study.

These 80 patients were further classified into nonsevere dengue (Group 1) and severe dengue (Group 2) by WHO classification of dengue 2009.[3] Therefore, patients with and without warning signs were included in group 1, and those with severe manifestations like severe plasma leakage, severe bleeding, and severe organ impairment were included in group 2. There were 50 patients in group 1 and 30 in group 2. Blood samples were collected from both groups for complete hemogram, liver function test, renal function test and cytokine levels. Blood samples collected for estimation of cytokine levels were centrifuged to separate the serum and then stored at -80 degree Celsius. Serum levels of all three cytokines (INF-γ, IL-6, and TNF-α) were measured using ELISA kits. All admitted patients were monitored daily till discharge or death in the hospital. Both the groups were compared on the basis of serum cytokine levels along with clinical, biochemical and radiological parameters.

Statistical analysis. Data were recorded using a structured proforma and managed on an excel sheet. All qualitative variables were compared with Chi-square test or Fisher's exact test. Quantitative variables with a normal distribution were compared using Student's t-test for two groups and ANOVA test with post hoc Bonferroni correction for the three-group analysis. Quantitative variables not following a normal distribution such as cytokine levels were compared using non-parametric test (Kruskal-Wallis test). A *p*-value of less than 0.05 (<0.05) was considered as significant. The analysis was done between Group 1 (nonsevere dengue, n=50) and Group 2 (severe dengue, n=30).

Results. Out of 80 positive patients, 37 patients were IgM antibody positive, and 43 patients were NS-1 antigen positive. The mean duration of fever in group1 and group 2 were 5.09 ± 1.7 days and 5.22 ± 2 days respectively. The most common symptoms apart from fever were headache (92%), followed by chills (84.1%), myalgia (74.6%), nausea (55%), vomiting (49%), retro-orbital pain (58%), arthralgia (39%) and rash (28%). Also, patients with severe dengue had more headache, myalgia, retro-orbital pain, abdominal pain, diarrhea, respiratory distress, oliguria and altered sensorium as compared to patients with nonsevere dengue (**Figure 1, 2**). The mean systolic BP (SBP) in group 2 (94 ± 11.0 mm Hg) was lower than the mean SBP in patients with group 1 (113 ± 09 mm Hg) (*p*-value <0.001). Similarly, mean diastolic pressure (DBP) in group 2 (50 ± 10.0 mm Hg) was significantly lower than mean DBP in group 1 (70 ± 11.0 mmHg) (*p*-value <0.001) (**Table 1**).

The most common clinical finding amongst all patients was positive tourniquet test (76%), followed by a rash (55.5%). The mean platelet count in group 2 (70364 ± 50431/ul) was lower than in group 1 (93460 ± 65000/uL), but this difference was not statistically significant. Mean AST levels in group 2 (451 ± 633.2 IU) were significantly higher than in group 1 (96.5 ± 157.4 IU, *p*=0.01). The mean serum ALT levels in group 2 (270.3 ± 334.25 I.U.) were also higher than in group 1 (60.16 ± 73.22 IU, *p* = 0.01). Bleeding manifestations were more in group 2 (83.3%) as compared to group 1 (46%). The most common form of bleeding manifestation was skin petechiae, seen in 36% of group 1 patients and 63.3% of group 2 patients. Gastrointestinal bleeding (hematemesis, melena, or hematochezia) was present in 8% of group 1 and 56.6% of group 2. One patient had iliopsoas bleed and required surgical intervention (**Figure 3**).

Tumor Necrosis Factor-α, Interleukin-6 and Interferon-γ levels were estimated in the serum of 80 patients from the sample collected on the day

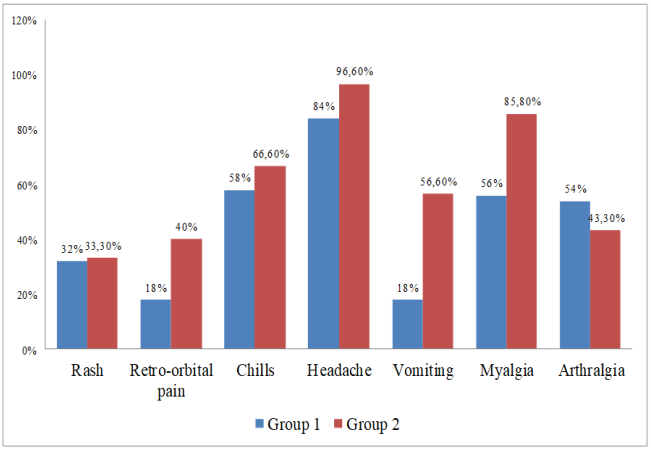

Figure 1. Frequency of symptoms in the two study groups.

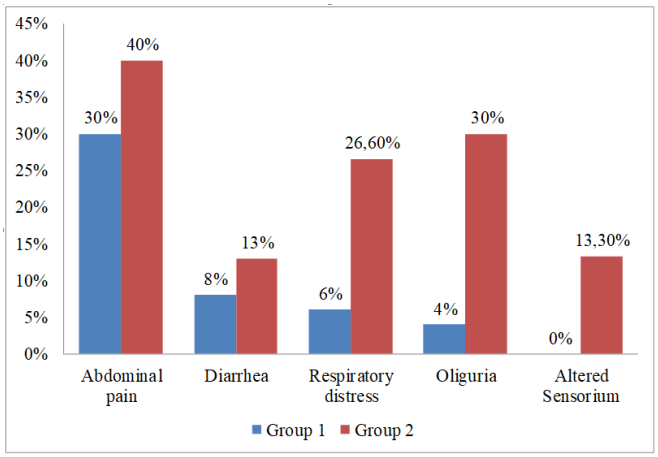

Figure 2. Frequency of symptoms in the two study groups.

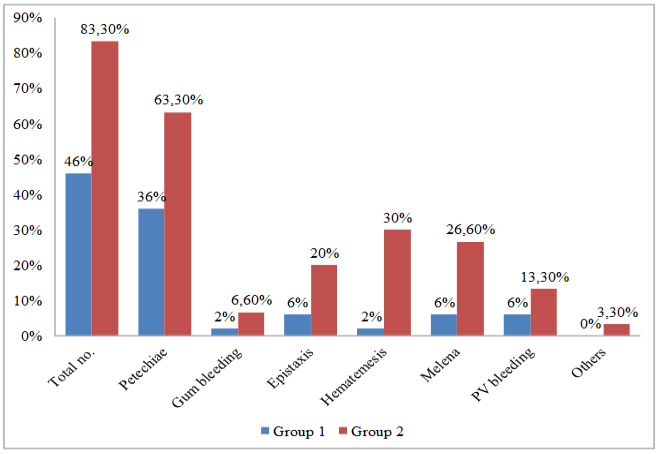

Figure 3. Distribution of various forms of bleeding manifestation among two groups.

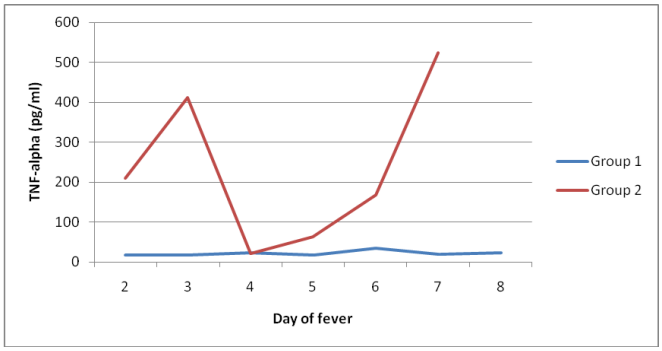

Figure 4. Line diagram showing median levels of TNF-α plotted against days of fever.

of the presentation. The median level of serum TNF-α in group 2 (62.5 pg/mL) was significantly higher than the median level in group 1 (20 pg/mL), (p=0.043). Similarly, the median level of serum IFN-γ in group 2 (10.25 pg/mL) was significantly higher than the median level in group 1 (8.5 pg/mL), (p=0.002). The median level of IL-6 was also higher in group 2 (29 pg/ml) as compared group 1 (14.2 pg/ml), but this result was not significant (p>0.05) (**Table 2**).

Further, the median levels of both TNF-α and IFN-γ were calculated for each day of fever according to the day of fever on presentation and sample collection. The serum levels of TNF-α were significantly higher on day 2-3 of illness, followed by a fall on day 4-5 and a late upsurge in group 2 as compared to group 1. Similarly, IFN-γ levels were also plotted across the day of fever and significant increase was noticed on initial days of

Table 1. Comparison of vital parameters among patients in the two groups.

Vital parameter	Group 1 (mean±SD)	Group 2 (mean±SD)	P-value
PR (beats/min)	88±12	105±10.2	0.001
SBP (mmHg)	113±9	94±11.0	0.001
DBP (mmHg)	70.7±11	50±10	0.001
RR (breaths/min)	17.4±1.9	19.6±2.82	0.001
Temp (ºF)	99.7±0.9	99.4±1.05	0.261

Table 2. Comparison of cytokine levels between the two groups.

Cytokine	Group 1		Group 2		p-value
	Mean	Median	Mean	Median	
TNF-alpha (pg/ml)	112.8 ± 213.1	20	213.17 ± 266.9	62.5	0.043
IFN-gamma (pg/ml)	11.8 ± 9.2	8.5	28.4 ± 56.2	10.25	0.002
IL-6 (pg/ml)	39.76 ± 54.4	14.2	61.46 ± 70.77	29	0.093

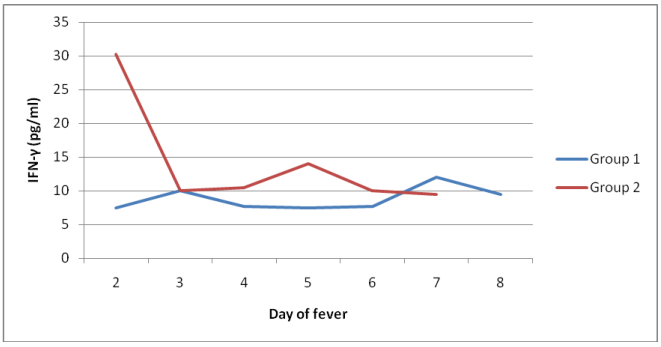

Figure 5. Line diagram showing median levels of IFN-γ plotted against days of fever.

presentation followed by a fall and a second rise in the levels in group 2 (**Figure 4, 5**). The decline in the mid-phase is not expected but may be attributed to hemodilution due to intensive intravenous fluid therapy.

Discussion. The most common presenting symptoms in our study were headache, myalgia, nausea, and vomiting. The observed frequencies of symptoms in our study are similar to those previously reported in the literature but with some notable differences.[4,5] Retro-orbital pain and arthralgias were infrequent in previous reviews, but in the present study, almost half of the patients had these symptoms. In our study, the mean platelet count in group 2 was significantly lower than the mean platelet count in group 1. Few studies have shown that low platelet counts are a predictor of dengue severity.[6,7] However, our study did not demonstrate any statistical significance between mean platelet count in these two groups.

Deranged liver function in dengue infection can be a result of the direct effect of the virus on hepatocytes or unregulated host immune response against the virus.[8] Mahmuduzzaman and colleagues showed that both AST and ALT were significantly raised in patients with DHF as compared to those with dengue fever and increase in AST was much higher than the increase in ALT.[9] Similarly, Pancharoen and coworkers also reported that levels of AST and ALT were significantly higher among patients with more severe disease.[10] In present study too, the difference in both AST and ALT levels in the two groups were significant.

Studies in the recent past have highlighted the role of cytokines and other biomarkers in the pathogenesis of severe dengue and have studied the utility of these biomarkers as risk factors.[11,12] It has been clearly demonstrated that the inflammatory response associated with deregulated cytokine production perform critical roles in the development of severe dengue.[13] The higher levels of cytokines like TNF- α, IL-1β, IFN-γ, IL-4, IL-6, IL-13, IL-7, and GM-CSF were associated with severe dengue fever in various studies.[14,15,16] In present study too, the levels of TNF-α and IFN-γ were significantly higher in severe dengue group (group 2). The elevated levels of cytokine in severe dengue make them good predictors of severity of dengue fever. Cytokine estimation at presentation can provide us a clue whether a patient is likely to develop severe manifestations of dengue or not. However, our study has certain limitations, like small sample size and the serotype of dengue virus was not studied. Further large prospective studies are warranted for better comprehension of the balance between circulating cytokines and their effect on the development of severe dengue.

Conclusions. Some cytokines like TNF-α and INF-γ may play a role in pathogenesis of severe dengue fever and can act as predictors of dengue severity.

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