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Research Article

Role of Pro-inflammatory IL-8 and Anti-inflammatory IL-10 Cytokines in Dengue Severity

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A B S T R A C T

Introduction: During dengue infection, cytokine levels may increase as various cytokines are released from infected inflammatory cells. This study was conducted to measure the levels of cytokines IL-8 and IL-10 in dengue patients and correlate them with dengue severity.

Material & Methods: A prospective study was conducted on febrile patients suspected of dengue fever, seeking medical care in our institute. 107 cases confirmed to have dengue fever (by NS1/ IgM ELISA) and 100 healthy individuals with age and sex matched, were included in the study. The clinical features of all patients were recorded, and cytokine levels of IL-8 and IL-10 were estimated by ELISA in the dengue patients and healthy controls.

Results: Out of 400 febrile patients suspected of having dengue fever, 107 (26.75%) cases were confirmed cases, of which 56 (52.3%), 20 (18.7%), and 31 (29%) were positive for only NS1 antigen, only IgM antibody, and both NS1 and IgM, respectively. Depending on the severity of the disease, 9 (8.5%) cases were classified as severe dengue cases while 98 (91.5%) as non-severe dengue fever. Mean levels (pg/ml) for IL-8 were 281.6 ± 76.6, 150.41 ± 55.9 and 75.4 ± 49.2 in severe dengue, dengue fever, and healthy controls respectively while for IL-10, the values were 219.4 ± 150.5, 38.9 ± 67.2, and 6.6 ± 0.65 among severe dengue cases, dengue cases, and healthy controls, respectively.

Conclusion: Mean level of cytokines IL-8 and IL-10 were significantly raised in severe dengue patients as compared to non-severe dengue patients and healthy controls, suggesting their role in causing severe disease and as a potential predictor for disease severity and fatal outcome.

Keywords: Dengue, Cytokines, IL-8, IL-10, ELISA

Introduction

Dengue fever, an emerging vector-borne disease, is a major public health problem, with an estimated 390 million infections occurring every year putting about 3.6 billion people at risk.[1] Infection with the dengue virus (DENV) results in a wide spectrum of disease ranging from self-limiting illness to full-blown dengue fever (DF) with or without warning signs, to severe life-threatening dengue shock syndrome (SD). Patients with DENV infection commonly presents with fever, headache, fatigue, nausea, chills, joint pain, and dizziness but in a cohort of patients, it may deteriorate to life-threatening severe dengue characterised by vascular leakage leading to shock, internal haemorrhage, and organ impairment resulting in death, if left untreated.[2]

Currently, 4 serotypes of dengue virus are identified to be in circulation viz. DENV1, DENV2, DENV3 and DENV4.[3] All four serotypes though serologically related, differ antigenically. Infection with any one of the four viral serotypes confers protective immunity against re-infection to only the same serotype, while subsequent infections with other serotypes result in manifestations of severe dengue due to an antibody-dependent enhancement (ADE) leading to cytokine storm.[4,5] However, some authors suggest that infection to dengue virus does not provide lifelong immunity and a person may be re-infected with the same serotype of the virus.[6]

The host immune response in DENV infection is marked by ADE which has been proposed to play a major role in the pathogenesis of severe dengue manifestations. During dengue infection, various kinds of cytokines are produced, which are mediated by both T helper 1 (Th1) and T helper 2 (Th2) cells. The level of these cytokines fluctuates with the severity of dengue infection, implying a vital role of these cytokines in the pathogenesis and severity of the disease.[7,8] While certain cytokines like tumour necrotic factor-α and interleukin-4, -5, -6 play a pro-inflammatory role in the pathogenesis of the disease, others like IL-10, -13, and interferon (IFN) have an anti-inflammatory role.9 Hence the present study was undertaken to detect the level of IL-8 and IL-10 in dengue fever and dengue haemorrhagic fever patients, with the aim that it will open up new avenues in understanding the association between pro and anti-inflammatory cytokines and their roles in the pathogenesis and subsequent clinical manifestations of dengue infections.

Materials and Methods

This study was carried out in the Department of Microbiology, SGT Medical College Hospital & Research Institute, Gurugram, Haryana from May 2019 to December 2020. The study included 400 clinically suspected cases of dengue fever who presented either to the emergency or OPD or were hospitalised and above 18 years of age. Patients excluded from the study were those who were already diagnosed as having enteric fever, malaria or any other infections, other than dengue. Demographic details, clinical history along with relevant investigations (haematological and biochemical) were meticulously recorded and maintained. All the patients in the study were classified into non-severe dengue cases (Group 1) which included patients with and without warning signs of dengue and severe dengue cases (Group 2) consisting of patients with severe manifestations like severe plasma leakage, bleeding, and organ impairment, in accordance with WHO classification.[10] Age and sex matched, and apparently disease-free persons (n = 100), mostly institutional volunteers were included in this study as healthy controls (Group 3).

Approximately 5 ml of blood sample was collected aseptically by venepuncture in a plain vacutainer and the serum separated was aliquoted into sterile storage vials of which one was used for confirmation of dengue fever and the other was stored for further analysis. Samples of all 400 patients suspected of dengue fever were subjected to a confirmatory test for dengue by detection of NS1 antigen and IgM antibodies in serum by enzyme-linked immunosorbent assay (ELISA) method. All the serum samples positive for dengue virus were stored at -80 degree Celsius for estimation of cytokine levels. Serum cytokine levels of Il-8 and IL-10 were measured on day 0 and day 5 for admitted patients and day 0 for OPD patients and control group by ELISA method (Day '0' being the day of the first presentation to the OPD/ IPD). All admitted patients were monitored daily till discharge or death in the hospital. Standards were included in each assay and standard curves obtained were used for the estimation of cytokine concentration. Institutional Human Ethics Committee approval was obtained before the start of the study and blood samples were collected only after obtaining informed written consent.

Statistical Analysis The data were analysed and the cytokines levels were represented as mean and standard deviation (mean ± SD). The mean difference of these values between the categories of dengue fever was compared using one–way ANOVA. The statistical significance of difference in the levels of IL8, IL10 between various groups (healthy, non-dengue, dengue, severe dengue) were analysed using post hoc test. P-value < 0.05 (0.05) was considered statistically significant.

Results

During the study period, a total of 400 samples were tested for dengue. Out of the total samples tested, 26.75% (n = 107) were positive for dengue. Among the dengue positive patients, 33.6% (n = 36) were admitted in the hospital

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and 66.3% (n = 71) were from out-patient departments. Majority of the cases tested for dengue were obtained in the year 2019 in the month of September, October, and November with the highest peak in November (Figure 1). Seropositivity for dengue was high in 18-30 years of age group (n = 50, 46.7%) followed by 31-40 years of age (n = 33, 30.8%). Percentage positivity in male and female patients suffering from dengue fever were 58% (n = 62) and 42% (n = 45) respectively, and the affected male: female ratio was found 1.37:1 in this study (Figure 2). Of the dengue positive samples, 81.3% (n = 87) tested positive for NS1 antigen whereas, 47.6% (n = 51) samples were positive for anti-dengue IgM antibody. Among the NS1 antigen-positive samples, 31 (35.6%) were also found to be positive for IgM antibody while 56 (64.4%) were negative for the antibody test (Table 1).

When the clinical presentation of the patients was analysed, the most common symptoms apart from fever were headache (87%), followed by nausea (72.4%), myalgia (65.3%), abdominal pain (57.1%), arthralgia (56.1%), vomiting (48%), rash (22%), and retro-orbital pain (21.4%). The patients presented the illness for a duration of 4.3 ± 1.8 (mean ± SD) days. On the basis of clinical manifestations and severity of the disease, 98 (91.5%) patients were classified as non-severe dengue patient (Group 1) while 9 (8.5%) patients were severe dengue patients (Group 2) as shown in Table 2. Patients with severe dengue had more headache, nausea, vomiting, myalgia, retro-orbital pain, abdominal pain, and arthralgia as compared to patients with non-severe dengue (Figures 3 and 4). The mean systolic BP (SBP) in Group 2 (106 ± 11.4 mm Hg) was lower than the mean SBP in patients of Group 1 (120 ± 11.7 mm Hg) (p-value < 0.05). Similarly, mean diastolic pressure (DBP) in Group 2 (59.6 ± 6.0 mm Hg) was significantly lower than mean DBP in Group 1 (74.7 ± 6.7 mm Hg) (p-value < 0.05). The mean platelet count in Group 2 (64888.9 ± 16021/ul) was lower than in Group 1 (106816 ± 73972.2/uL). Mean ALT levels in Group 2 (401.4 ± 239.8 IU) were significantly higher than in Group 1 (77.7 ± 73.1 IU, p = 0.01). The mean serum AST levels in Group 2 (217.6 ± 92.9 IU) were also higher than in Group 1 (68.6 ± 70.4 IU) (p- value < 0.05) (Table 3).

Table 1.Distribution of Viral Serology (IgM detection) by ELISA in Clinically Suspected Dengue Cases

Result of IgM Antibody Test	Result of NS1 Antigen Test		Total
	Positive (n = 87)	Negative (n = 313)	
Positive, n (%)	31 (35.6)	20 (6.4)	51
Negative, n (%)	56 (64.4)	293 (93.6)	349

Table 2.Distribution of Dengue Positive Cases according to Severity of Disease

Cases	Frequency	Percentage
Non-severe dengue	98	91.5
Severe dengue	9	8.5
Total	107	100

Table 3. Comparison of Vital Parameters among Dengue Positive Patients in the Two Groups i.e., Non-severe Dengue Patients (Group 1) and Severe Dengue Patients (Group 2)

Vital Parameters	Group 1 Mean ± SD	Group 2 Mean ± SD	Statistical Comparisons
SBP (mm Hg)	120.4 ± 11.7	106.7 ± 11.4	< 0.05
DBP (mm Hg)	74.7 ± 6.7	59.6 ± 6.0	< 0.05
RR (breath/min)	17.5 ± 3.7	19.4 ± 3.2	NS
Temp (deg C)	102.3 ± 1.8	102 ± 1.4	NS
Platelet count	106816.3 ± 73972.2/UL	64888.9 ± 16021/UL	< 0.05
ALT	77.7 ± 73.1	401.4 ± 239.8	< 0.05
AST	68.6 ± 70.4	217.6 ± 92.9	< 0.05
SpO2	97.0 ± 2.0	97.2 ± 1.5	NS

NS: Not significant, p-value > 0.05.

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Table 4. Association of Cytokine Levels with Dengue Severity among the Various Study Groups

Cytokine (pg/ml)	Group 1 Non-severe Dengue Patients		Group 2 Severe Dengue Patients		Group 3 Healthy Control	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
IL-8	150.4 ± 55.9	150.4	281.6 ± 76.6	280	75.4 ± 49.2	53.2
Il-10	38.6 ± 64.8	11.8	219 ± 150.5	145	6.6 ± 0.65	3.1

Table 5.Median Level of Cytokines at 5 Days Interval among the Dengue Positive Patients (n = 36)

Study Group	No. of Day	IL-8	IL-10	Statistical Comparisons
Group 1 (Dengue fever)	Day 0	150	11.8	< 0.05
	Day 5	59	7.8	< 0.05
Group 2 (Severe dengue)	Day 0	280	145	< 0.05
	Day5	78	45	< 0.05

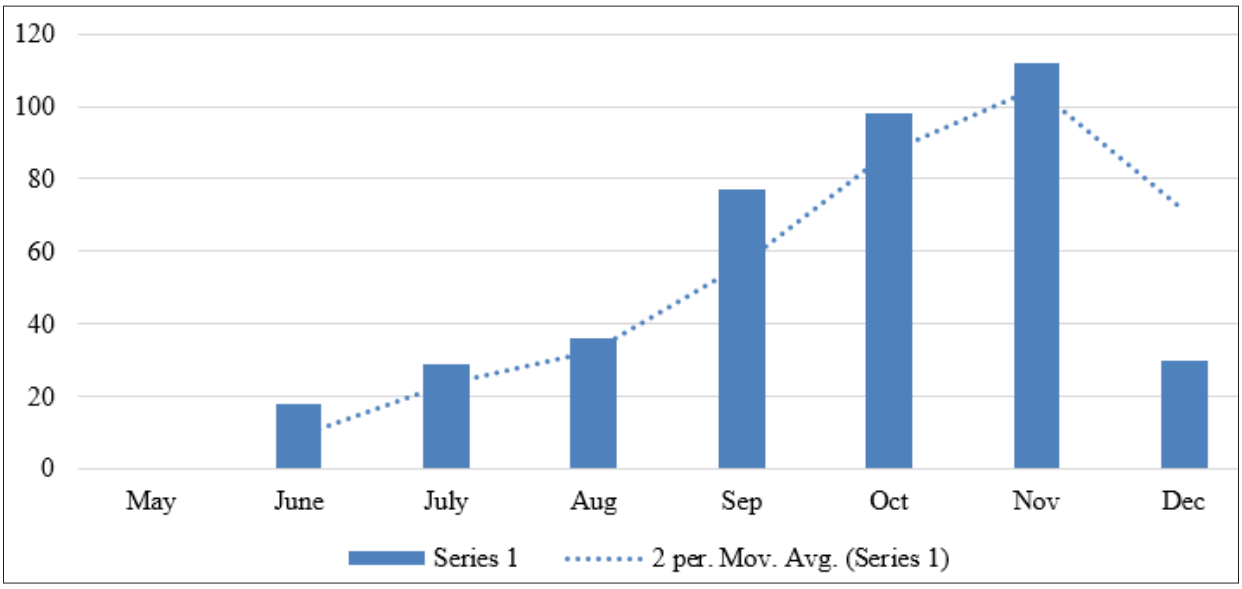

Figure 1.Month-wise Distribution of Laboratory Confirmed Positive Dengue Fever Cases

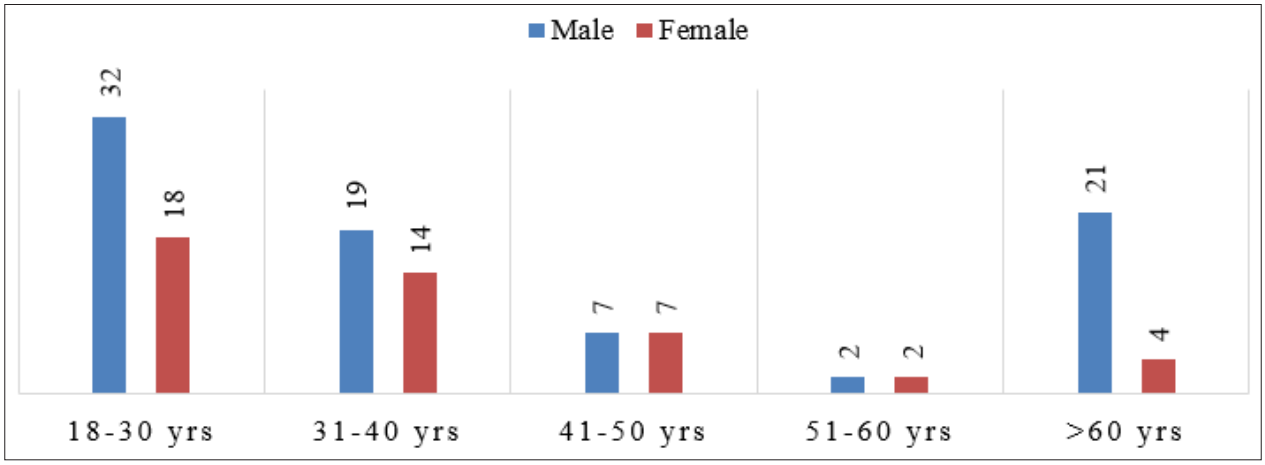

Figure 2.Distribution of Positive Patients according to the Age Groups

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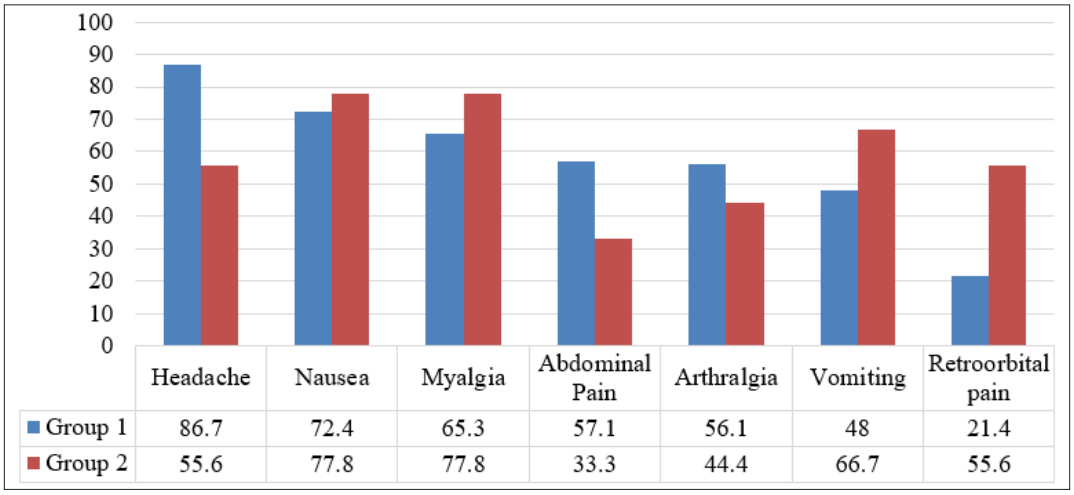

Figure 3.Frequency of Symptoms in the Two Groups

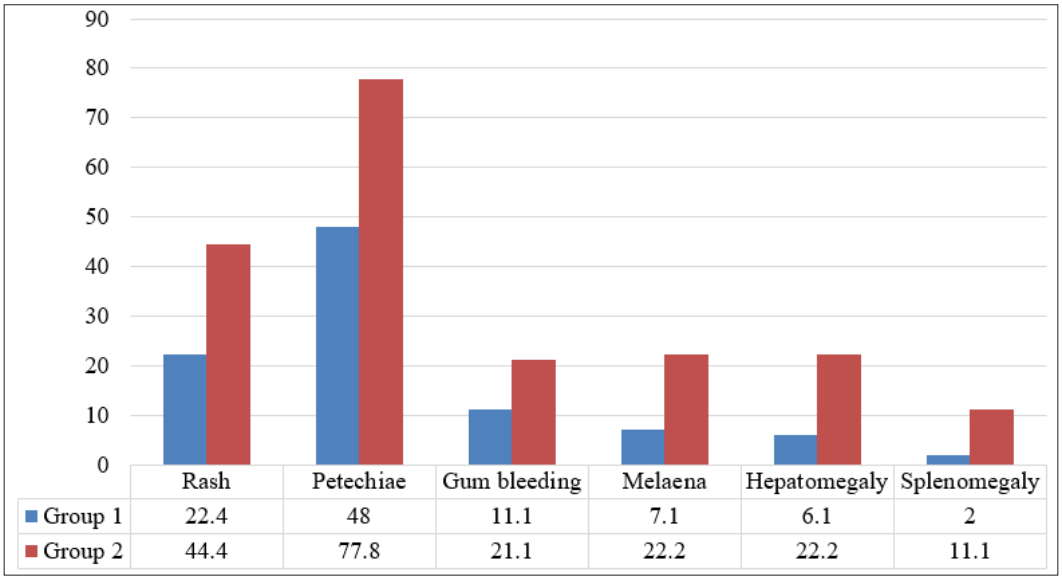

Figure 4.Distribution of Clinical Signs in the Dengue Positive Patients (n = 107)

Serum interleukin-8 and interleukin-10 levels were estimated in all the dengue positive patients (n = 107) on the day of the presentation (day 0). The median level of serum IL-8 in Group 2 (280 pg/mL) was significantly higher than the median level in Group 1 (150.4 pg/mL), ($p \leq 0.05$) and Group 3 (53.2 pg/mL). Similarly, the median level of serum IL-10 in Group 2 (145 pg/mL) was significantly higher than the median level in Group 1 (11.8 pg/mL), ($p \leq 0.05$) and Group 3 (3.1 pg/mL) (Table 4). Serum concentrations of IL-8 and IL-10 of 36 admitted patients were further measured on the 5th day of their admission to perform intra-group analysis. The levels of both the cytokines showed a significant decline in values over the five days duration with a p-value of < 0.05 in both the groups (Table 5).

Discussion

For over two centuries, India is endemic for dengue infection. During dengue infection, cytokines level may increase as various cytokines are released from infected inflammatory cells. In severe dengue fever, more viruses enter the macrophages and multiply within them, which leads to immune enhancement.[11] This leads to an increased release of cytokines (cytokine storm) which play an important role in pathogenesis and clinical manifestations of severe dengue infections. With this hypothesis in mind, the present study was undertaken to understand the role of cytokines in clinical manifestations of dengue virus infection.

In our study, 26.75% of cases were positive for dengue virus infection as confirmed serologically for the presence of dengue virus NS1 antigen or IgM antibodies. Dengue seropositivity in our study is in concordance with a study by Savargaonkar et al. who reported 27.7% seropositivity of dengue infection from Delhi in 2018.[12]

On analysis of data obtained on a monthly basis, seasonal variation of disease was observed. The observation of seasonal transmission of dengue virus infection with increased activity in monsoon and post-monsoon season

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in the present study was in accordance with the reported patterns of seasonal variations of dengue transmission by various authors.[13] This may be explained by the fact that stagnant water sources following heavy rainfall favour breeding of the mosquito vector resulting in an increased incidence of post-monsoon dengue infections, thereby maintaining the mosquito vector population throughout the year.[13] Effective control and preventive measures should be implemented diligently during monsoon periods to avoid water stagnation after the initial bouts of rainfall and also at the end of the season.

In the present study, seropositivity for dengue fever was higher in the age group of 21-30 years (46.7%), followed by 30.8% in the age group of 31-40 years. These findings are consistent with other Indian studies. Gupta et al.[14] and Chakravarti and Kumar et al.8 also reported maximum cases in the age group of 21-30 years. The reason may be that active adults are involved in more outdoor work, so they face greater chances of getting infected. The vector Aedes aegypti mosquito is mostly active 2 hours after sunrise and several hours before sunset, hence they primarily bite during daytime. A higher prevalence of dengue infection was noted among males (57.9%) than females (42.1%). The affected male to female ratio was 1.37:1 which correlates well with other Indian studies.[15-17]

The results of our study show that the frequency of clinical manifestations such as myalgias, arthralgias, retro-orbital pain, rash, and haemorrhage, were more frequently observed in patients with severe dengue infection than those with dengue fever. Besides predicting the chance of increased severity in a case, the clinical usefulness of this difference is questionable although the findings are comparable with other studies by various authors.

In our study, the mean platelet count in Group 2 was significantly lower than the mean platelet count in Group 1. Although low platelet counts are a predictor of dengue severity,[18] our study did not demonstrate any statistical significance between mean platelet count in these two groups. Deranged liver function in dengue infection may result due to the direct effect of the virus on hepatocytes or unregulated host immune response against the virus.[19] Severe dengue infection results in hepatic injury which leads to elevated levels of transaminases and manifests as abdominal pain.[19] Mahmuduzzaman et al. reported significant rise in both AST and ALT in DHF patients when compared to patients with dengue fever and also noted that AST level increased much higher than ALT level.[20] Similarly, Pancharoen et al. and Shivkar RR et al. reported that levels of AST and ALT were significantly higher among patients with more severe disease.[21,22] In the present study, the difference observed in both AST and ALT levels in the two groups were significant.

Cytokine estimation during the first presentation of the patient can provide an important clue about the likelihood of the development of severe clinical manifestations of dengue infection. In the present study, the levels of IL-8 and IL-10 were found to be significantly higher in severe dengue (Group 2) as compared to Group 1 and healthy controls. Our study showed that levels of both the cytokines progressively decreased over a period of 5 days, as the patients recovered, implying an increased production of these cytokines early in the course of the disease. However, we were able to estimate cytokines levels at day 0 (day of first presentation to the hospital) and day 5 for IPD patients in only 36 patients during their stay at the hospital, while only on day 0 for OPD patients as most of the patients did not turn up for a later visit or follow up on day 5.

Dengue virus infection results in the release of various cytokines from the virus-infected lymphocytes, monocytes, and mast cells. Thus, it is expected to note an increase in the cytokine levels during dengue infections. Various studies in the past have highlighted the role of cytokines and other biomarkers in the pathogenesis of dengue virus infections.[8] The inflammatory response associated with deregulated cytokine production plays a critical role in the pathogenesis and eventually in the development of severe dengue infections,[23,24] hence by identifying such predictors, we can focus on those cases who are more likely to develop severe dengue infections, thus reducing the overall morbidity and mortality due to dengue virus infection.

Conclusion

It was observed that levels of cytokines IL-8 and IL-10 correlated with disease severity suggesting that these cytokines can be used as an early marker to predict the progression of the severity of the disease. Early prediction of the severity of the disease will help detect cases requiring continuous monitoring and thereby help us devise necessary treatment plans and precautions in advance. However, our study has certain limitations, like small sample size, no correlation with different serotype of dengue viruses involved and roles of other cytokines which could also have been implicated in DF pathogenesis were not evaluated.

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Conflict of Interest: None

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