Original Research

Estimation of IL 6 levels in dengue fever and its correlation with disease severity: A cross sectional study

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ABSTRACT:

Introduction: Dengue infection (DV) is a common vector-borne disease which can cause a myriad of features. 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. **Aim**: To measure IL-6 levels in serum samples of DV2-infected patients and its correlation with disease severity. **Material and methods:** A total of 80 cases were included in the study which were divided into group 1 comprising of 40 cases of DF and group 2 comprising of 40 cases of DHF. Serum IL-6 levels were estimated using ELISA kit. **Results**: Mean age of DF and DHF group patients was 19.4 and 28.9 years, respectively. Gender distribution showed that Group 1 included 40.4% males and 59.6% females, while group 2 had , 52.5% were males and 47.5% females. The most common symptoms apart from fever were headache (95%), followed by chills (82.5%), myalgia (77.5%), nausea (50%), vomiting (42.5%), abdominal pain (40%), arthralgia (27.5%), rash (25%), thrombocytopenia (22.5%), bleeding (10%), and conjunctival congestion (8.7%). mean level of IL-6 expression was 162.43 pg/mL in group 1 and 612.36 pg/mL in group 2. The difference in IL-6 expression within both the study groups was statistically also significant. ($P < 0.05$) **Conclusion**: 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.

Key words: Dengue , Flavivirus, interleukin-6, prognosis.

INTRODUCTION:

Dengue infection (DV) 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. Dengue virus (DV) is transmitted through mosquito bite and is prevalent in over 100 tropical and subtropical countries with about millions of people at risk .[1]

It is an arboviral disease in humans caused by a Flaviviridae family member, a single-stranded positive-sense RNA virus. South East Asia Region (SEAR) and western Pacific region bear nearly 75% of the current global disease burden due to dengue.[2] DHF has emerged as the most important arbovirus disease in man in the last two decades.

There are four antigenically distinct cross-reactive serotypes of DV (DV1–4) with no cross protective immunity, and transmitted mainly by female mosquito of Aedes aegypti. This infection initially is mostly asymptomatic or produces a mild self-limiting acute febrile illness, dengue fever (DF). This further leads to dengue hemorrhagic fever (DHF) characterized with plasma leakage and hemorrhagic manifestations progressing towards dengue shock syndrome (DSS). Various neurological manifestations such as encephalitis, myelitis, Guillain-Barré syndrome (GBS), hypokalemic paralysis, and myositis are also well reported in dengue, but pathogenesis is still controversial.

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.[3]

Existing literature suggests three interacting components such as deregulation of cell-mediated immunity, antibody-dependent enhancement (ADE) and the complement system working together to produce a potentially life-threatening state during DV infection.[5-7]

A deep understanding of DV pathogenesis is necessary for its therapeutic strategies development. IL-6 is one of the top candidates as a prognostic marker for severe DV infection. Hence the present study was aimed to measure IL-6 levels in serum samples of DV2-infected patients and its correlation with disease severity.

MATERIAL AND METHODS:

This case-control study was carried out in Department of Medicine Hind Institute of Medical Sciences Ataria Sitapur U.P. with Institutional Ethics Committee approval and written informed consent from all participants before the conduct of any study-related procedure. Demographic and clinical data were collected from all enrolled cases with detailed medical history and physical examination.

All patients of DV infection with fever and various neurological manifestations during the study period were included in the study. From these patients, total 80 (40 patients of DF and 40 patients of DHF) were selected who were Serologically DV2 positive and their serum were collected. Controls (n=40) were obtained from age- and sex-matched otherwise healthy persons undergoing spinal anesthesia in various surgical departments. These patients of control group were serologically negative for HCV, HBV, HCMV, EBV, JEV, HIV and DV,

Sera obtained from study samples were separated by centrifuging the whole blood at 3500 r/min for 8 min and stored at –80 °C until subjected to detection of IL-6 protein employing DRG IL-6 ELISA kit EIA-4640, according to the manufacturer's instruction. To check the accuracy and authenticity of experiment, negative and positive controls were employed with ELISA kit.

STATISTICAL ANALYSIS:

Statistical analysis was performed using SPSS version 20 for windows (Chicago, IL, USA). Categorical variables were expressed as percentages. Student unpaired t-test was performed for obtaining p values and $P < 0.05$ were considered statistically significant.

RESULTS:

A total of 80 cases were included in the study which were divided into group 1 comprising of 40 cases of DF and group 2 comprising of 40 cases of DHF. Mean age of DF and DHF group patients was 19.4 and 28.9 years, respectively. Gender distribution showed that Group 1 included 40.4% males and 59.6% females, while group 2 had , 52.5% were males and 47.5% females. Various demographic findings of the study sample are tabulated in table 1.

TABLE 1: DEMOGRAPHICS PARAMETERS OF STUDY GROUP (DF AND DHF PATIENTS)

DEMOGRAPHIC PARAMETERS	GROUP 1 (n=40)	GROUP 2 (n=40)
MEAN AGE (years)	19.4	28.9
GENDER (%) MALE FEMALE	40.4% 59.6%	52.5% 47.5%
MEAN DURATION OF FEVER	8.2 ±1.40	10.2±1.1
TLC (cells x 10^3)	4.2±2.1	4.8±3.4
HEMOGLOBIN (gm/dl)	11.4±2.4	12.1±1.2
HEMATOCRIT (%)	34.6±3.4	38.6±4.6
PLATELET COUNT (10^6 cells/µl)	1.3±0.81	1.14±1.2
SERUM CREATININE (mg/dl)	1.38±1.4	1.41±1.1
SERUM UREA (mg/dl)	40.24±21.1	41.4± 18.2
ALP levels (IU/L)	232.8±201.2	276±212.1
ALT levels (IU/L)	94.4±78.1	182.2±220.4
AST levels (IU/L)	102.5±98.4	191.4±184.2
TOTAL BILIRUBIN (mg/dl)	1.42±1.3	1.49±0.52

TLC: Total leukocyte count; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

The most common symptoms apart from fever were headache (95%), followed by chills (82.5%), myalgia (77.5%), nausea (50%), vomiting (42.5%), abdominal pain (40%), arthralgia (27.5%), rash (25%), thrombocytopenia (22.5%), bleeding (10%), and conjunctival congestion (8.7%). Headache, chills, fever, rash and nausea were found to be common among both groups but other severe finding like abdominal pains, thrombocytopenia, bleeding, respiratory distress and congestion were more seen in group 2 patients.

FIGURE 1: DISTRIBUTION OF CLINICAL SYMPTOMS WITHIN STUDY GROUPS
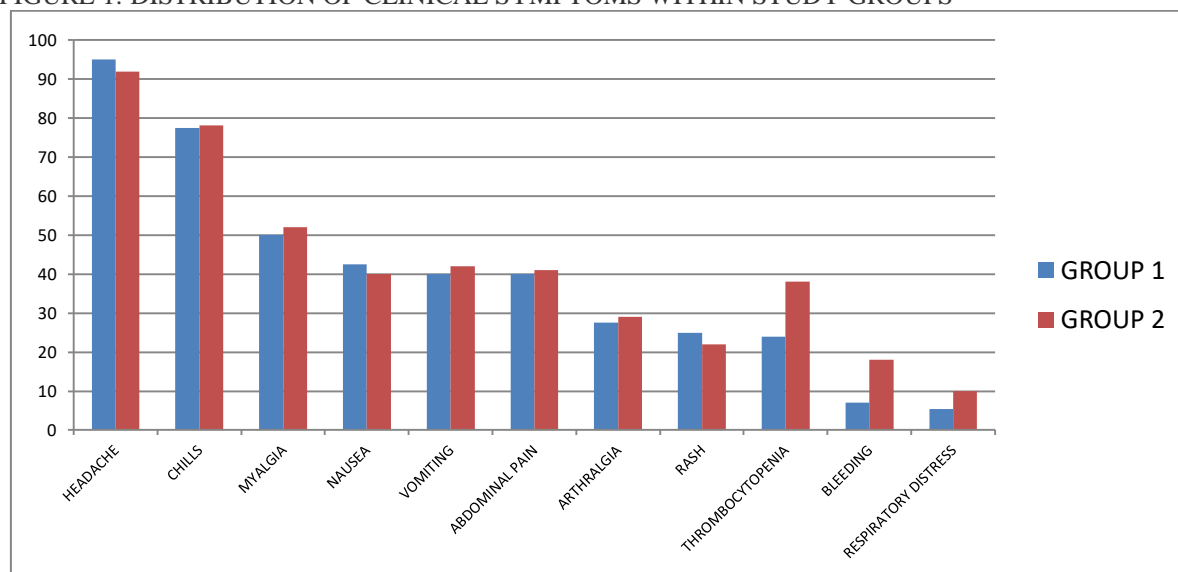
On evaluation of serum IL6 levels we observed an elevated IL-6 expression in both groups. Healthy patients which comprised our control group had a mean level of IL-6 expression of 6.52 pg/mL, while in both study groups (DF and DHF groups), mean level of IL-6 expression was 162.43 pg/mL in group 1 and 612.36 pg/mL in group 2. The difference in IL-6 expression within both the study groups was statistically also significant. (P < 0.05)

FIGURE 2: COMPARISION OF MEAN IL6 LEVELS AMONG CONTROL AND STUDY GROUPS.
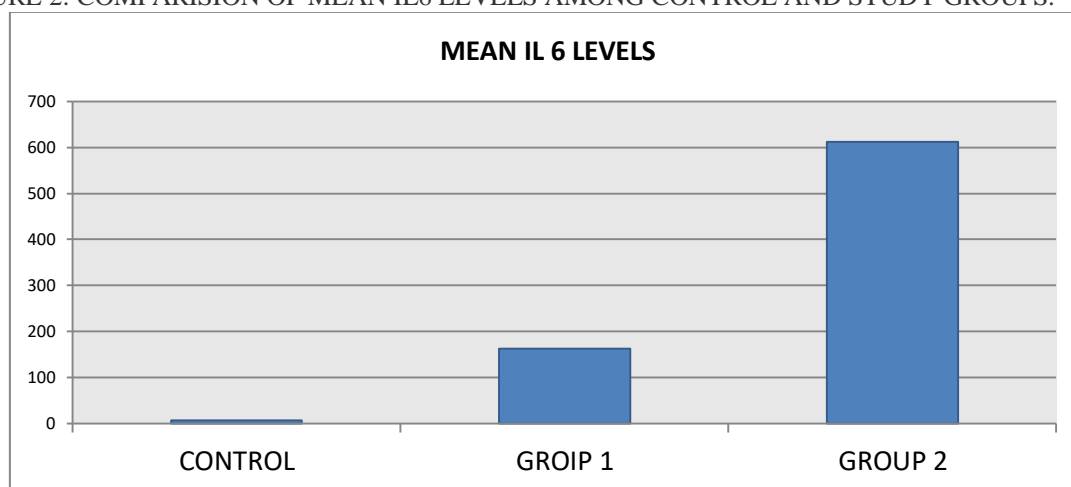
DISCUSSSION:
Studies in the recent past have highlighted the role of cytokines and other biomarkers in the pathogenesis of severe dengue and have studied the utility of these biomarkers as risk factors. It has been demonstrated that the inflammatory response associated with deregulated cytokine production perform critical roles in the development of severe dengue.

The aim of this study was to show the role of a pro-inflammatory marker (IL-6) in the pathogenesis of DV infection. IL-6 is a multifunctional cytokine that acts on different target cells to induce a variety of biological responses involving immune regulation and signal transduction. The most common symptoms apart from fever were headache followed by chills, myalgia, nausea, vomiting, abdominal pain, arthralgia,

rash , thrombocytopenia, bleeding and conjunctival congestion. The observed frequencies of symptoms in our study are similar to those previously reported in the literature but with some notable differences[8,9] though Retro-orbital pain and arthralgias were infrequent in previous reviews.

Results of this study showed that mean level of IL-6 expression was significantly higher in study groups in comparison to controls. Further we observed mean IL 6 levels of 162.43 pg/mL in group 1 (DF) which was significantly lower in comparison to 612.36 pg/mL levels in group 2 (DHF). Thus we observed sequential changes of serum IL-6 level and clinical progression of the disease from DF to DHF. In similarity to our study Sergio IC et al[10] and Fariha Kanwal et al [11] also reported similar findings.

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With regard to dengue disease, the most significant finding of a study showed a shift from the predominant Th1-type response observed in cases of DF to the Th2-type in severe cases of DHF grade IV. Increased serum levels of IL-4, IL-6 and IL-10 were observed mainly in cases of DHF grades III and IV. In contrast, the levels of IFN-γ and IL-2 were highest in cases of DF and low in DHF grade IV. The cytokine levels to increase first were IL-2, IL-6, IFN-γ and TNF-α while IL-4, and IL-10 tended to emerge during days 4–8 of the illness.[12]

In yet another study by Mehta VK et al[13] it was shown that the level of IL-6 and IL-8 was found to be higher in CSF and serum samples in DF. Besides, the pro-inflammatory role of IL-6 in experimental autoimmune neuritis and Guillain-Barré syndrome (GBS), it is shown that it may have additional neuroprotective effects by reducing TNF, IL-1, and promoting SC proliferation and remyelination. IL-8 along with IL-1ra (receptor alpha) mediates the recruitment and activation of lymphocytes and monocytes expressed in the CSF of GBS patients.[14] Both IL-6 and IL-8 along with other cytokines released by tubular epithelia in response to reduced total or regional blood flow to the kidney enhances inflammation and cell injury.[15] This supports the observation of raised serum level of cytokines like IL-6 in our study.

CONCLUSION:

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.

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