

Elevated Plasma Interleukin-10 levels in Dengue Hemorrhagic Fever

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ABSTRACT

Aim: To measure the levels of plasma interleukin-10 in patients suffering from dengue hemorrhagic fever (DHF) between 4-7 days of onset of disease.

Methods: In this cross sectional analytical study, a total of 50 diagnosed patients of DHF, 15-50 years of age of either sex, presented between 4 to 7 days of onset of disease were included. Plasma IL-10 levels were measured by using standard commercial enzyme-linked immunosorbent assay.

Results: The mean plasma levels of IL-10 were observed to be elevated in all the subjects suffering from DHF but significantly higher in those patients who presented on 5th and 6th days of disease onset. More number of patients (65.2) were afebrile who presented on day 5th and 6th (representing defervescence phase), as compared to those who presented on 4th and 7th days of disease onset (35%).

Conclusions: It is concluded that IL-10 levels are markedly elevated during the defervescence phase of dengue hemorrhagic disease, providing its potential role in the pathogenesis of disease.

Keywords: Interleukin-10, cytokines, dengue hemorrhagic fever.

INTRODUCTION

Dengue virus (DENV) infection, the most important emerging arthropod-borne viral disease is caused by any of the four serotypes of dengue viruses (DENV-1-4)¹. Each of the four dengue virus serotypes is capable of causing a spectrum of diseases ranging from asymptomatic infection to undifferentiated fever, mild dengue fever (DF) to a severe dengue hemorrhagic fever (DHF) which may progress to dengue shock syndrome (DSS)². Although DF and DHF look alike in the first few days of disease but they are two different clinical conditions from the beginning³. DHF is classified on the basis of severity which varies from mild (WHO grade I and II) to severe (WHO grades III and IV) grades⁴.

Despite extensive studies, the pathogenesis of DHF is still not fully understood^{5,6,7,8}. It is widely accepted that dengue virus infections induce a strong inflammatory response by producing different inflammatory cytokines⁹. Interleukin-10 (IL-10), a known anti-inflammatory as well as immunosuppressant cytokine is found to play an important role in DHF¹⁰. Evidences show that DHF/DSS occurs around the third to seventh day of illness during the course of disease and IL-10 also tends to appear in the late phase (defervescence phase) of dengue illness, suggesting the potential

role of this cytokine in the pathogenesis of DHF^{11,12,13}. This study was undertaken to determine the levels of plasma IL-10 in patients with DHF in the population of Pakistan.

MATERIALS AND METHODS

With the approval of Ethical Review Board and the consent of the participating patients, this study was undertaken in the department of Physiology, FPGMI, SZMC, Lahore in collaboration with Services Hospital, Mayo Hospital and Jinnah Hospital, Lahore. A total of 50 diagnosed patients of DHF, 15-50 years of age of either sex, presented between 4 to 7 days of onset of disease were included in this study. On the basis of medical records and history, participants with known conditions, affecting plasma levels of IL-10, such as Dengue fever, Chikungunya, Malaria, Enteric fever, Hepatitis B&C, Diabetes mellitus, Malignancies, Sepsis and Tuberculosis were excluded from the study. On the day of presentation, three milliliters (ml) of blood sample was drawn from each subject. Plasma was separated from blood sample by centrifugation, aliquoted, labeled and kept frozen at -20°C until analyzed for IL-10 levels. Plasma IL-10 levels were measured by using standard commercial ELISA based kit, manufactured by Diaclone (France). The procedure was done in NHRC, FPGMI, Shaikh Zayed Hospital. The data was arranged and analyzed by using SPSS version 19.0.

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RESULTS

This study included 50 DHF patients, out of which, 13 presented on 4th day, 15 on 5th and 6th day each and 7 on 7th day of onset of disease. There were 38 males and 12 females with mean±SD age of 31.55±11.42 years and 27.75± 9.11 years respectively. When, number of patients with history of fever was noted, it was seen that 46 (92%) patients with mean± SD of 29.85±10.72 were found afebrile. But only 4 (8%) patients with mean± SD of 39.75±10.69 were presented with history of fever (Table 1). It was observed that more number of afebrile patients (65.2%,) were presented on day 5th and 6th as compared to 4th and 7th days (35%), with a p-value 0.006 (Table 2). For comparison of plasma IL-10 levels in all the four days of fever, Kruskal–Wallis test was applied.

Table 1: Distribution of febrile and afebrile patients according to age (years)

Age (Yrs)	Fever	Afebrile	Total
15-24	0	18(39%)	18(36%)
25-34	1(25%)	12(26%)	13(25%)
35-44	1(25%)	11(24%)	12(24%)
≥45	2(50%)	5(11%)	7(14%)
Total	4(100%)	46(100%)	50(100%)
Mean±SD	39.75±10.69	29.85±10.72	30.64±10.95
Median	41	27.50	28
Q1	29	20	21.50
Q3	49.24	35.25	36.75

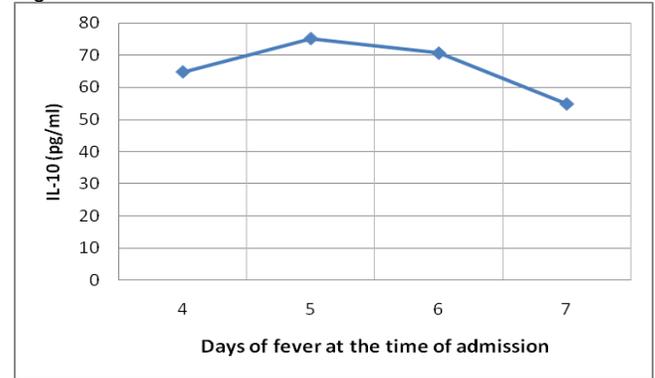
Table 2: Distribution of febrile and afebrile patients by day of fever at the admission time (between 4-7 days of onset of disease)

Day fever at admission time	Fever	Afebrile	Total
4thday	4(100%)	9(19.5%)	13(100%)
5 th day	0	15(32.5%)	15(100%)
6 th day	0	15(32.5%)	15(100%)
7 th day	0	7(15.2%)	7(100%)
Total	4(100%)	46(100%)	50(100%)

χ²: 12.38 P-value: 0.006 p-value < 0.05 significant

The mean plasma levels of IL-10 were observed to be elevated (Normal levels:1.25-15.6 pg/ml) in all the subjects but significantly higher in those patients who presented on 5th and 6th days of disease onset. The mean± SD for plasma IL-10 levels are 64.84±18.91, 75.20±18.00, 70.66±18.27 and 54.85±20.17 on 4th, 5th, 6th and 7th days of disease respectively and were significantly different with a p value < 0.001 (Figure.1).

Figure 1: Plasma IL-10 levels



DISCUSSION

To our knowledge, this is the first study in Pakistan with the aim to investigate the levels of plasma IL-10 in patients suffering DHF. This study found significantly raised levels of plasma IL-10 in DHF patients.

Our results are in consistent with a number of studies that suggested that dengue virus infection induces a strong antigenic inflammatory response, resulting in deregulated and increased cytokine production, among which IL-10 is known as a major regulatory cytokine⁹. According to many previous studies, it has been observed that 5th and 6th days of fever are the most vulnerable days to develop shock during the course of disease¹⁴. Moreover, peak levels of plasma IL-10 have been noted on the same days of fever. Since severe manifestations of dengue disease and characteristically the disease outcome mostly develop around the time of defervescence phase. In the present study, it is noted that the maximum number of patients presented on 5th and 6th days of fever were afebrile at their time of admission (representing defervescence phase) along with significantly high levels of plasma IL-10¹⁵.

Although IL-10 is a known anti inflammatory cytokine, but due to its immunosuppressive ability, it can down regulate antigen presenting cell response and suppression of intracellular antiviral response. It might be possible that increases in IL-10 concentrations were associated with an increased probability of DHF. These overall raised levels of plasma IL-10 establish a necessary basis for its role in pathogenesis is in agreement with previous

reports^{7,16,17}. A study carried out by Gurugama et al (2010) correlated the elevated levels of IL-10 with disease severity¹⁸. These results were supported by another study conducted by Ubol et al (2010), in which they found elevated levels of IL-10 in DHF and highlighted its role in pathogenesis¹⁹.

Our results are in contrast to those studies who found no significant association of increased plasma IL-10 levels with the disease pathogenesis. According to Sierra et al (2010), the increased levels of IL-10 could be in apparent contradiction with the pathogenic role attributed to this cytokine, taking into consideration that raised IL-10 levels have been reported in DHF patients²⁰.

CONCLUSION

We found the high levels of plasma IL-10 levels in patients suffering from DHF between 4 to 7 days of onset of disease. It is concluded that significantly high levels of plasma IL-10, especially on the fifth and sixth days of fever provide its potential role in the pathogenesis of the dengue disease.

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