ORIGINAL ARTICLE

Interleukin-10 as a Marker of Disease Progression in Dengue Hemorrhagic Fever

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Abstract

Objective: To evaluate the plasma interleukin-10 (IL-10) levels in patients suffering from dengue hemorrhagic fever between 4 to 7 days of onset of disease and 24 hours after the first sample, to find out the association of plasma IL-10 levels with the outcome.

Study Design: Analytical study.

Place and Duration of Study: All major hospitals of Lahore, Pakistan, from August to November 2012.

Methodology: Participants included 50 registered patients of dengue hemorrhagic fever (DHF) aged between 15 - 50 years. Plasma IL-10 concentrations were measured on above stated day. Outcome was described as recovery and shock. Platelet count and hematocrit percentages were also recorded. Statistical analyses were done using SPSS version 19. A p-value ≤ 0.05 was considered significant.

Results: Plasma IL-10 levels were found to be raised in DHF patients and were associated with fatal outcome (p=0.004). In recovered DHF patients, plasma IL-10 levels decreased after 24 hours (mean 26.54 ±16.03 pg/ml) as compared to admission time (mean 74.39 ±61.69 pg/ml) but in case of DHF patients suffering from shock, plasma IL-10 was found to be higher after 24 hours (mean 87.69 ±7.77 pg/ml) as compared to levels at admission time (mean 42.56 ±28.09 pg/ml). ROC curve analysis revealed a change (30 units pg/ml) of plasma IL-10 concentration, within 24 hours of admission, raised from the base line to be 105 times more critical for shock in DHF patients (100% sensitivity and 71.4% specificity, p < 0.001).

Conclusion: Elevated plasma IL-10 is a potential predictor of disease severity and fatal outcome in DHF patients.

Key Words: *Interleukin-10. Immunopathogenesis. Cytokines. Dengue hemorrhagic fever. Outcome.*

INTRODUCTION

Dengue virus infection, caused by four antigenically related but genetically distinct dengue (DEN) virus serotypes of genus *Flavivirus*, is increasingly being recognized as a major health, social and economic problem in the tropical and sub-tropical areas worldwide.[1] Currently, WHO estimates 390 million dengue infections per year of which 96 million (67-136 million) manifest dengue fever (with any severity of disease).[2] Although infection with one serotype induces a lifelong protective immunity against that serotype (known as primary infections) but provides only transient protection with a heterologous serotype, resulting in secondary infection leading to a greater disease severity.[3] Infection can range from asymptomatic condition to mild acute febrile illness to classical DEN fever and/or to severe dengue hemorrhagic fever (DHF) which may progress to dengue shock syndrome (DSS), characterized by shock or death.[4]

Substantial gaps remain in the basic understanding of the pathogenesis of dengue disease.[5] It has been shown by epidemiologic and clinical associations that both host immunological and viral factors determine the severity of the disease.[6] It has been recognized that the interplay of inflammatory response and deregulated cytokine production in DHF may play key roles in protection or increased disease severity as well as development of severe clinical manifestations. Interleukin-10 (IL-10), known as a major regulatory cytokine of inflammatory responses, has been found to be significantly higher in patients suffering from DHF.[7] Previous studies showed that DHF/DSS occurs around the 3rd to 7th day of illness during the course of disease and IL-10 also tends to appear in the later phase (defervescence phase) of dengue illness, suggesting its correlation with the disease severity.[8,9] Moreover, by processing dual nature both as anti-inflammatory as well as immune suppressant, it plays an important role in the pathogenesis of DHF.[10,11]

In present study, the potential predictive role of plasma interleukin-10 as a marker of disease progression in

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Received: March 06, 2015; Accepted: October 26, 2015.

DHF patients was evaluated. The objective of this study was to evaluate the plasma interleukin-10 (IL-10) levels in patients suffering from dengue hemorrhagic fever between 4 to 7 days of onset of disease and 24 hours after the first sample, to find out the association of plasma IL-10 levels with the outcome.

METHODOLOGY

This analytical study was undertaken in collaboration with all the major hospitals of Lahore, Pakistan after taking approval from respective Ethical Review Boards of those institutions. The population under consideration consisted of fifty (50) diagnosed patients of DHF, aged 15 - 50 years, of either gender; all presenting between day 4 to 7 of onset of disease. The sample size was determined by using 5% level of significance and 81% power of test, with expected plasma IL-10 levels of 120 ±50 picogram/milliliter (pg/ml) and 130 ±40 picogram/milliliter (pg/ml) on the day of admission and after 24 hours of the first sample, respectively.

From each registered patient, fulfilling the inclusion criteria, an informed consent along with basic demographic data, medical history, physical examination and laboratory investigations were recorded on the predesigned proforma. Participants with known comorbid conditions such as dengue fever, Chikungunya, malaria, enteric fever, Congo hemorrhagic fever, hepatitis C, hepatitis B, diabetes mellitus, malignancy and tuberculosis were excluded from the study.

Paired blood samples (first at the time of admission and second after 24 hours of the first sample) were obtained from each patient. Plasma was aliquoted, labelled and kept frozen at -20°C until analyzed for IL-10 levels by commercial ELISA kit (Diaclone, France). Normal IL-10 levels were taken as 1.25 - 15.6 pg/ml.[12] Laboratory work was done at National Research Council Laboratory, Postgraduate Medical Institute, Lahore.

Data was arranged and analyzed by using SPSS version 19.0. The data regarding plasma IL-10 levels was deviating from normality so for comparison of plasma IL-10 levels, at admission time and 24 hours later for all days (4 - 8 days) of fever. Kruskal-Wallis test was applied and for comparison between two independent groups, Mann-Whitney-U test was used. Effect of plasma IL-10 on the outcome of patients was related by binary logistic regression. Receiver operating Characteristic (ROC) curve was used to evaluate the diagnostic performance of plasma IL-10 at optimal cut-off point. Fisher's exact test was used for determining association between two qualitative variables. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

This study enrolled 38 males and 12 females with mean age of 31.55 ±11.42 and 27.75 ±9.11 years, respectively. Significantly greater number of afebrile patients (p=0.006) presented on day 5th and 6th as compared to 4th and 7th day (Table I).

Regarding outcome, 43 patients were recovered from DHF, whereas 7 patients suffered shock. The trend showed that out of 7 cases who suffered shock, maximum number of patients, i.e. 4 (57.1%) and 2 (28.6%) followed shock on day 5th and 6th, respectively (Table II).

During all the 4 (4 - 7) days of fever, the mean plasma levels of IL-10 were observed to be elevated in all the subjects. In recovered cases, the levels of plasma IL-10 were decreased after 24 hours (mean 26.54 ±16.03 pg/ml) as compared to elevated levels at the time of admission (mean 74.39 ±61.69 pg/ml, Figure 1). However, in shocked patients, the levels were markedly increased after 24 hours (mean 87.69 ±7.77 pg/ml) from the admission time levels (mean 42.56 ±28.09 pg/ml, Figure 2).

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

Day of fever at the admission time	Fever		Afebrile		Total		X^2	p-value
	n	(%)	n	(%)	n	(%)		
4th	4	(8%)	9	(18%)	13	(26%)	12.38	0.006*
5th	0	(0%)	15	(30%)	15	(30%)		
6th	0	(0%)	15	(30%)	15	(30%)		
7th	0	(0%)	7	(14%)	7	(14%)		
Total	4	(8%)	46	(92%)	50	(100%)		

**p- value ≤ 0.05 significant*

Table II: Distribution of patients by day of fever at the time of admission and outcome (between 4 - 7 days of onset of disease).

Day of fever at the time of admission	Recovered		Shocked		Total	
	n	%	N	%	n	%
4thday	12	(27.9%)	1	(14.3%)	13	(26%)
5thday	11	(25.6%)	4	(57.1%)	15	(30%)
6thday	13	(30.2%)	2	(28.6%)	15	(30%)
7thday	7	(16.3%)	0	(0%)	7	(14%)
Total	43	(100%)	7	(100%)	50	(100%)

Binary logistic regression was applied to observe the effect of IL-10 on the outcome of patients 24 hours later, which gave the odds of DHF as 1.072 (1.023 - 1.124 with 95% CI, p=0.004) showing the positive effect of high levels of plasma IL-10 on the fatal outcome.

To analyze the diagnostic performance of the plasma IL-10, an average receiver-operating characteristic (ROC) curve, by plotting the sensitivity versus the 1- specificity, was constructed. The Area Under curve (AUC), closer to 1 indicated greater discriminatory power. The optimum cut-off point, calculated as the maximum value of sensitivity multiplied by specificity was 30 pg/ml (100% sensitivity and 71.4% specificity). It showed that when the change in plasma IL-10 concentration increased by 30 pg/ml within 24 hours of admission, the sensitivity to detect shocked cases was 100%; and at this point, the specificity was 71.4%. (Table III, Figure 3).

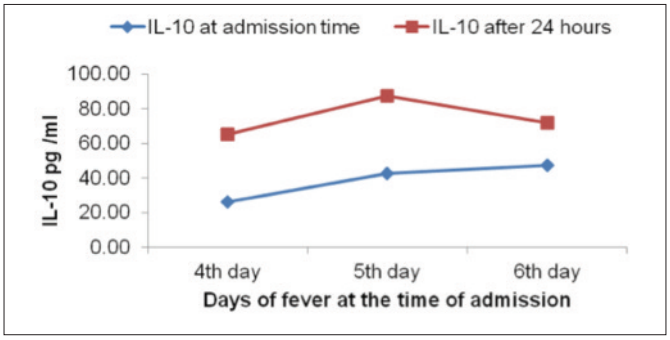

Figure 1: Average levels of plasma IL-10 at the time of admission and after 24 hours of recovered patients (4 - 7 days).

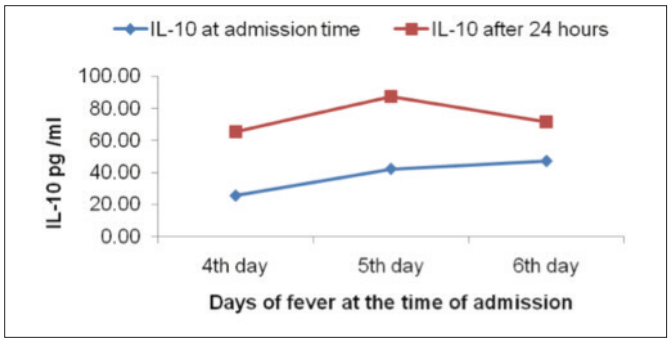

Figure 2: Average levels of plasma IL-10 at the time of admission and after 24 hours of shocked patients (4 - 6 days).

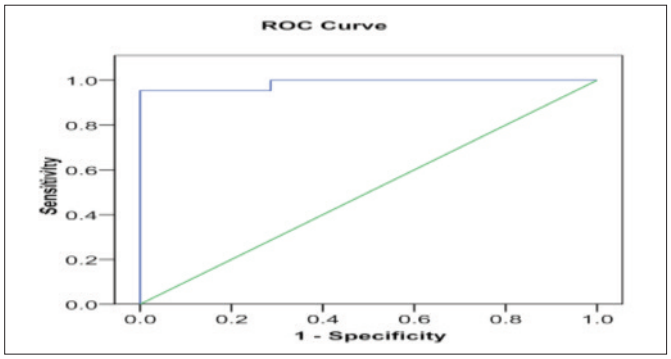

Figure 3: ROC curve for cut-off point of plasma IL-10 change (difference at the time of admission and after 24 hours in 4 - 7 days).
Area under curve (AUC) = 0.987, CI 95% = 0.961-1.013, Sensitivity (%) = 100%, 1- Specificity (%) = 71.4%.

DISCUSSION

To the authors' knowledge, this was the first study in Pakistan with the aim to investigate the prognostic role of 1L-10 in patients suffering from dengue hemorrhagic fever (DHF). This study demonstrated significantly elevated levels of plasma IL-10 in DHF patients and its positive effect on the fatal outcome.

Dengue fever and outcome pathogenesis is poorly understood. Dengue hemorrhagic fever may be the result of dysfunction of host immune system.[12] Previous studies suggested that a strong antigenic inflammatory response, resulting in cytokine storm, is seen in dengue virus infection.[10] Interleukin-10, an immunomodulatory Th2 cytokine, is known as a major regulator of inflammatory responses by exerting anti-inflammatory effects through several mechanisms.[13]

It was noticed that DHF/DSS occurs around the 3rd to 7th day of illness during the course of disease.[14] In the present study, it was observed that the maximum number of patients, who had suffered shock, presented on 5th and 6th day. They were afebrile at their time of admission (representing their defervescence phase) with significant high levels of plasma IL-10. Since severe manifestations (plasma leakage) and outcome of dengue disease mostly develops around the time of defervescence phase,[11,15] so, significantly elevated level of plasma IL-10 in the defervescence phase sheds light on the role of IL-10 in the pathogenesis of DHF. The correlation of IL-10 with disease severity could be attributed to its immunosuppressant nature besides its anti-inflammatory role. It can also down regulate antigen presenting cell response and intracellular antiviral response, helping in the facilitation of dengue virus replication.[16] In the present study, overall raised levels of plasma IL-10, by establishing a necessary basis for its role in pathogenesis, are in agreement with previous

Table III: Association of plasma IL-10 (on the basis of change in plasma IL-10 concentration, at the time of admission and 24 hours later ≥ 30 or ≤ 30) with outcome.

Change in plasma IL-10 Conc.	Outcome		Total	Fisher's exact test
(at the time of admission and after 24 hours)	Shocked	Recovered		(p-value)
Change in plasma IL-10 Conc. ≥ 30 pg/ml	5	2	7	< 0.001
Change in plasma IL-10 Conc. ≤ 30 pg/ml	0	43	43	
Total	5	45	50	

***p-value ≤ 0.001*

reports.[10] Other studies carried out by Gurugama *et al*. and Ubol *et al*. correlated the elevated levels of IL-10 with the disease severity.[17,18]

It was found that rapid measurement of IL-10 (approximately within one week of fever) may serve as a useful marker for early detection of potential DHF cases. These results were supported by another study conducted by Brasier *et al*. which nominated plasma IL-10 as a key biomarker for early prediction of DHF as elevated plasma IL-10 levels were associated with an increased probability of DHF.[19]

It was also found that elevated levels of IL-10 correlate positively with fatal outcome of patients suffering from DHF. Similarly, the study conducted by Brooks *et al*. correlated the elevated levels of IL-10 with fatal outcome of patients suffering from DHF.[20]

Contrarily, these results were in contrast to studies which found no significant association of increased plasma IL-10 levels with severity of disease.[21,22] Differences in sample size and detection technique of IL-10, followed by these researches, may be the reason behind these results.

Longitudinal studies with serial measurements of IL-10 as well as other cytokines would be helpful in better understanding of the disease pathogenesis. In future, further research is needed to study the role of genetic polymorphism of IL-10 and other cytokines to identify risk groups in a dengue exposed population.

CONCLUSION

Significantly elevated plasma interleukin-10 is a potential predictor of disease severity and clinical outcome in DHF patients.

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