Original Article

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Assessment of Serum Interleukin-10 as a Marker of Severity in Dengue Virus Infection: A Cross-sectional Study

Microbiology Section

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ABSTRACT

Introduction: Interleukin-10 (IL-10) plays an important role in dengue pathogenesis, reflecting an immunosuppressive function that causes Interferon (IFN) resistance, followed by impaired immune clearance and a persistent infectious effect for acute viral infection.

Aim: To evaluate and compare IL-10 levels as a potential indicator of disease severity in dengue infection.

Materials and Methods: The present cross-sectional study was conducted in the Department of Microbiology, Government Thoothukudi Medical College, Tamil Nadu, India, from March to September 2023. A total of 315 clinically suspected dengue patients in the age group of 1 to 80 years were subjected to IgM Enzyme Linked Immuno Sorbent Assay (ELISA). All samples that tested positive were considered confirmed dengue cases and were included in the study. Out of which 43 samples were positive for dengue (Group 1, n=28) and (Group 2, n=15). Group 1 included Dengue Fever (DF) with or without warning signs, Group 2 included severe dengue and Group 3 included 30 healthy volunteers. IL-10 was measured using the Diaclone ELISA kit. Patient's demographic profiles and laboratory parameters were collected. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) v19.0 with Pearsons Chi-square test and One-way Analysis of Variance (ANOVA). A p-value <0.05 was considered significant.

Results: Out of 315 samples, 43 were confirmed by Dengue IgM ELISA. Out of the total 43 positive samples, 24 (55.8%) were paediatric patients and 19 (44.2%) were adult patients. Twenty (46.5%) were males and Twenty Three (53.5%) were females, respectively. Out of 43 samples, 28 (65.1%) in Group 1 and 15 (34.9%) were in Group 2. There was a significant association (p<0.05) between the IL-10 and the dengue severity. The mean IL-10 in severe dengue cases (222±80.7 pg/mL, median=130) was significantly (p<0.0001) raised as compared to non-severe dengue (IL-10: 51.4±21.37, median=35) and healthy controls (4.05±0.45, median=3.5).

Conclusion: Early prediction of the severity of the disease will help in better management, ultimately benefiting the patients. The present study suggests that IL-10 may serve as an indicator for identifying patients with severe dengue and those with or without warning signs, highlighting the urgent need for a marker that reflects endothelial damage.

Keywords: Anti-inflammatory cytokines, Enzyme-linked immunosorbent assay, Interferon resistance

INTRODUCTION

Dengue Virus (DENV) is a positive-sense, single-stranded Ribonucleic Acid (RNA) virus that is spread by the bite of *Aedes aegypti* and is a member of the Flaviviridae family. Dengue is an emerging public health challenge that is endemic to the Indian subcontinent. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue-endemic countries [1]. Dengue viral infections can have a varied presentation, ranging from mild Dengue Fever (DF) to severe Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), which can result in mortality if not treated early. Currently, four serotypes of the DENV have been identified to be circulating in humans. DENV1, DENV2, DENV3, and DENV4 are serologically related but differ antigenically [2]. The pathological basis of DF is a rapid increase in the levels of cytokines and other chemical mediators that lead to severe manifestations of DHF, such as plasma leakage, shock, and bleeding [3]. According to World Health Organisation (WHO) Guidelines 2009, dengue is categorized into two groups: non-severe dengue or mild disease with or without warning signs and severe dengue [4].

During dengue infection, different types of cytokines are generated, and their levels vary with disease severity. Following infection, activated monocytes, macrophages, and dendritic cells produce a range of proinflammatory cytokines, such as IL-6, IL-8, Tumour Necrosis Factor-alpha (TNF-α), and IFN-γ. These cytokines play a role in endothelial dysfunction, capillary leak syndrome, and coagulopathy in severe dengue [5]. T-Helper-1 (Th-1) cells secrete IFN-γ, IL-2, and TNF, whereas Th-2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 suggesting an important role of these cytokines in the pathogenesis and severity of the disease. Tumour Necrotic Factor-α (TNF-α) and IL-4, IL-5, IL-6 act as pro-inflammatory cytokines while IL-10, IL-13, and IFN act as anti-inflammatory cytokines. A disturbance in the balance of IL-10 with inflammatory cytokines such as IL-6 and IL-8 was noted in patients with haemorrhagic manifestations [6]. IL-10 is a cytokine that affects inflammation and immunoregulation in a variety of ways [3]. In contrast, cytokines that reduce inflammation, particularly IL-10, have two functions. IL-10 protects tissues and reduces excessive inflammation; however, it may paradoxically accelerate the course of dengue. IL-10 inhibits the production of proinflammatory cytokines and impairs antigen presentation by dendritic cells and macrophages, potentially delaying viral clearance [7,8]. IL-10 overexpression has been linked to reduced CD4+ and CD8+ T-cell proliferation, inhibition of macrophage activation, and suppression of Th1-mediated responses, all of which may lead to immune dysfunction during the critical phase. It has been suggested that IL-10 may serve as a predictive indicator of illness severity, in addition to reflecting the degree of immune activation. The present study compares IL-10 levels among patients with non-severe dengue, severe dengue, and healthy controls. The present study analysed demographic trends (age, sex) in relation to dengue positivity and assess the association between IL-10 levels with dengue severity and disease outcomes. Therefore, there is a crucial need for a reliable marker that reflects endothelial damage and helps determine the severity of dengue infection. The present study aimed to evaluate the role of IL-10 as an indicator in assessing disease

severity in dengue infection. The primary objective of the study was to compare IL-10 cytokine levels in patients with non-severe dengue, severe dengue, and healthy controls. The secondary objectives of the study were to analyse demographic trends (age, sex) in relation to dengue positivity and to assess the association between IL-10 levels with dengue severity and disease outcomes.

MATERIALS AND METHODS

The present cross-sectional study was conducted from March to September 2023 at the Department of Microbiology, Govt. Thoothukudi Medical College, Thoothukudi, Tamil Nadu, India. Demographic details, clinical history along with relevant investigations complete blood count {Total Leucocyte Count (TLC), platelets, and haematocrit}, Liver Function Tests (LFT), coagulation profile (PT, PTT, and INR) and serum electrolytes were recorded. The study was conducted with the approval of the Institutional Ethics Committee under IEC no. 02/2023-10.

A total of 315 samples were tested for dengue out of which 43 were confirmed as dengue positive using IgM dengue ELISA. Forty- three dengue positive samples (group 1: DF with and without warning signs, group 2: severe Dengue) and group 3: 30 healthy volunteers. The present study was a time-bound cross-sectional study conducted from March to September 2023, including all eligible dengue patients during that period.

Inclusion criteria: All inpatients in the age group of {(1- 12 years (Paediatric) and 13-80 years (adolescents and adults)} clinically suspected of DF were subjected to dengue IgM ELISA test. All patients positive for dengue IgM ELISA were considered confirmed dengue cases and were included in the study.

Non-severe dengue group: DF with or without warning signs; severe dengue group: Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

Healthy control group who had not had a fever or other sickness in the previous three months. Confirmation of dengue negative status through serological testing Dengue IgM ELISA. Cytokine levels were also tested in healthy individuals of comparable ages who did not exhibit any clinical signs of infection in order to establish a baseline for reference [9].

Exclusion criteria: Patients excluded from the study were those who were already diagnosed as having enteric fever, malaria, leptospirosis or any other infections other than dengue.

Study Procedure

Samples were obtained from clinically suspected DF cases after informed consent using standard blood collection techniques. On the day of admission, the history, clinical symptoms, and laboratory tests of every patient with a confirmed case of dengue were documented in the proforma. The following laboratory tests were used to grade dengue patients: complete blood count {TLC<5000/mm^3 for leucopenia, platelets <1 lac/mm^3 and haematocrit}, Liver Function Test (LFT), coagulation profile (PT, PTT, and INR) and serum electrolytes.

About 5 mL of blood sample was collected in a plain vacutainer, centrifuged, and serum was separated [10]. Samples were subjected to an ELISA test for DENV by detection of Dengue IgM antibodies in serum using NIV Dengue IgM Capture ELISA Kits, National Institute of Virology, Pune, India (Batch no. 23-099). The sera which tested positive for dengue IgM was then subsequently tested for levels of IL-10. Blood samples collected for estimation of cytokine levels were centrifuged and the separated serum was stored at -80 degrees celsius [10]. Based on WHO 2009 criteria, all the enrolled patients were categorised into non-severe dengue (with or without warning signs) and severe dengue [4].

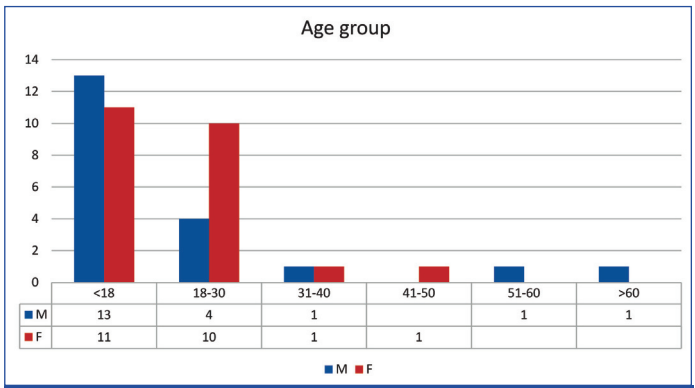
Serum levels of IL-10 were measured using commercial ELISA kits according to the manufacturer's instructions (Diaclone kit, France). The Standard diluent reconstitution gave a stock solution of volume 400pg/mL of IL-10. The reconstituted standard was mixed gently and serial dilutions of the standard were directly made on the assay plate to provide the concentration range from 400-12.5 pg/mL. The absorbance was then read on a spectrophotometer using 450 nm as the primary wavelength and optimally 620 nm as the reference wavelength. The standard solution is a recombinant human IL-10 protein with a known concentration, which is then serially diluted to create a standard curve for the quantitative measurement of IL-10 in the patient serum samples. Standards are included in each assay and a standard curve obtained was used for the estimation of cytokine concentration [11]. IL-10 levels were categorised into two groups: Moderate (0-50 pg/mL) and high/very high (51-100 pg/mL) based on thresholds used in previously published studies assessing cytokine levels in dengue [5].

STATISTICAL ANALYSIS

The IL-10 values were derived based on their Optical Density (OD) values and those were compared with the standards to compute the value in pg/mL. IL-10 values and dengue severity were analysed using Pearson's Chi-square test and the significance was calculated. Data were entered in Microsoft Excel and analysed using the SPSS 19.0. The Pearson's chi-square test was applied and a p-value <0.05 was considered significant. The comparison of mean IL-10 levels between groups was conducted using One-way ANOVA to assess the differences among the three groups (non-severe dengue, severe dengue, and healthy controls).

RESULTS

During the study's duration, 315 suspected dengue patient samples were received in the serology lab. Out of 315 samples, 43 were confirmed dengue positive samples by IgM dengue ELISA. Seropositivity for dengue was high in 18-30 years among the adult age group (n=14, 32.6 %) [Table/Fig-1].



[Table/Fig-1]: Distribution of age among study population.

Percentage positivity in male and female patients suffering from DF was 20 (46.5%) and 23 (53.5%) respectively. The male-to-female ratio was approximately 1:1.15. No statistically significant association was observed between sex and dengue positivity (p > 0.05). According to WHO guidelines 2009, Dengue is divided into two groups: group 1: Non-severe dengue or Mild disease with or without warning signs and group 2: Severe dengue includes DHF and DSS [4]. Out of 43 confirmed cases of dengue included in the study, 28 (65.1%) had non-severe dengue (group 1) and 15 (34.9%) patients had severe dengue (group 2) as depicted in [Table/Fig-2].

Out of the total 43 positive cases, 24 (55.8%) were paediatric patients and 19 (44.2%) were adult patients in [Table/Fig-3]. All the patients included in the study had a platelet count of less than 100,000/cu.mm, the minimum count being 17,000/cu.mm and a maximum of 100,000/cu.mm with a mean value of 55,000/cu.mm.

Cases	Frequency	%
Non-severe dengue	28	65.1%
Severe dengue	15	34.9%

[Table/Fig-2]: Distribution of dengue positive cases according to severity of disease.

The platelet count was recorded at the time of admission. IL-10 values and dengue severity were analysed using Chi-square test and the significance was calculated. There was a significant association ($p<0.05$) between the IL-10 and the dengue severity i.e., patients with severe dengue or dengue with warning signs had significantly higher number of IL-10 levels [Table/Fig-4].

One-way ANOVA was used to compare mean IL-10 levels among patients with non-severe dengue, severe dengue, and healthy controls. The analysis revealed a statistically significant difference in IL-10 levels across the groups ($F=254.72$, $p<0.0001$), indicating a strong association between elevated IL-10 levels and disease severity. Mean IL-10 levels were highest in severe dengue cases (222±80.7 pg/mL, median=130), followed by non-severe dengue (51.4±21.37 pg/mL, median=35), and lowest in healthy controls (4.05±0.45 pg/mL, median=3.5) [Table/Fig-5].

Age (years)	Non severe dengue		Severe dengue	
	Dengue Fever (DF)	Dengue with warning sign	Dengue haemorrhagic fever	Dengue shock
Paediatrics	8	12	3	1
Adults	5	3	9	2

[Table/Fig-3]: Distribution of age according to severity of disease.

IL-10	Non-severe dengue		Severe dengue	Chi-square statistics	p-value
	DF	DF with warning	DHF/ DSS		
Moderate (0-50)	10	11	1	18.289	<0.0001
High and very high (51-100)	3	4	14		
Total	13	15	15		

[Table/Fig-4]: Association between Interleukin-10 (IL-10) and dengue severity. Chi-square test is applied

IL-10 Cytokines (pg/mL)	Non-severe dengue	Severe dengue	Healthy controls	F value	p-value
	Mean±SD				
	51.4±21.37	222±80.7	4.05±0.45	254.72	<0.0001

[Table/Fig-5]: Association of cytokines levels with disease severity using One-way analysis of variance (ANOVA).

DISCUSSION

Dengue has become a global public health concern, especially in most tropical and subtropical countries. DENV infection is unique in that infection with one serotype results in lifelong immunity specific to that serotype. However, secondary infection with a heterogeneous serotype frequently causes severe illness through a mechanism known as Antibody-Dependent Enhancement (ADE), which is in contrast to protection or neutrality against other serotypes [9]. In the present study, seropositivity for DF was higher in the age group of <18 years (55.8%) followed by the age group of 18-30 (32.6%) in [Table/Fig-1]. These findings are consistent with studies of Mogha M et al., Sharma M et al., Dinkar A et al., [10-12]. It was found that an increased frequency of dengue infection was observed in the age group of 18-30 (32.6%) among the adults whereas in the age group <18, it was 24 (55.8 %). It was observed by Mandal SK et al., higher prevalence under the age group of 18-40 years (67.30%) whereas over 40 years it was 32.70 percent [13]. Prajapati H et al., reported that dengue was more frequent in the 20-40 years age group (39.4% of total cases) followed by <20 years age group [14]. This may be due to involvement in outdoor activities. A higher prevalence of dengue infection was noted among females (53.5%) than males (46.5%). The study by Prajapati H et al., examined 125 cases of dengue and found that the male predominance was not appreciably greater than the female predominance [14]. In the present study, distribution of dengue positive cases according to severity of disease among the 43 positive samples for DENV infection was analysed as indicated in [Table/Fig-2]. The results showed that non-severe dengue infection is the most common type of dengue infection in the population, with 65.1% (28/43) of the samples representing non-severe dengue infection and 35.9% (15/43) representing severe dengue infection. It is comparable to Rosenberger KD et al., who reported 84.4% (1463/1734) of patients with non-severe dengue and 15.6% (271/1734) of patients with severe dengue [15]. In a study by Mogha M et al., a total of 40 dengue-positive serum samples were analysed according to the severity of DENV infection in group 1 and it discovered that 90% of the samples represented non-severe dengue infection, while 10% of the samples represented severe dengue infection [10].

There was a significant association ($p<0.05$) between the IL-10 and the dengue severity i.e., patients with Severe dengue or dengue with warning signs had significantly higher number of IL-10 levels [Table/Fig-4] were similar to that found in the study of Pandey N et al., (2015) [16]. IL-10 levels are elevated in severe dengue and may assist in identifying patients at risk for severe disease or warning signs. The median level of serum IL-10 in group 2 (130 pg/mL) was significantly higher than the median level in group 1 (35 pg/mL) and healthy controls (3.5pg/mL) [Table /Fig-5]. It was found by Tsai TT et al., Saishruti G *et al*, Flores Mendoza LK et al., and Abhishek KS et al., that higher levels of IL-10 were detected in DHF/DSS patients compared with DF patients [17-20].

In Puc I et al., study, the mean S.D. of severe dengue patients was found to be greater than that of non-severe dengue patients and healthy controls [21]; 1.22±1.40, respectively, with a significant p-value. One study done by Lee YH et al., emphasises that elevated IL-10, up to day 7 of fever onset, stood out as a candidate prognostic marker for severe dengue [22]. In 2017, a study by Meena AA et al., there was a notable rise in the cytokine IL-10 in patients with secondary DENV infection in Western India as compared to primary [23].

Regular evaluation of IL-10 levels in confirmed dengue infections might improve risk assessment and target early treatment. Future perspectives include appropriate multivariate analysis to validate IL-10 as a biomarker and larger multicenter trials evaluating IL-10 patterns throughout illness phases is needed. To get a better result, other cytokines including TNFα and IL-6 must be studied.

Limitation(s)

The present study is limited by the fact that it was a time-bound, single-centre cross-sectional research. Also during the febrile period, IL-10 levels were only assessed once.

CONCLUSION(S)

DENV infection results in the release of various cytokines from virus-infected lymphocytes, monocytes, and mast cells. Elevated serum IL-10 levels are significantly associated with dengue severity and may reflect the immune response during infection. Early prediction of the severity of the disease will help the treating physicians start treatment and better manage the patients, ultimately benefiting them. According to the current study, IL-10 levels are significantly higher in severe dengue and may serve as a supportive marker in identifying patients with warning signs or severe disease.

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