International Journal of Contemporary Pediatrics
Manoharan R et al. Int J Contemp Pediatr. 2018 May;5(3):1109-1114
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20181552

Original Research Article

Cytokine IL-12, Dengue in children: analysis of a unique relationship

Rakesh Manoharan, Umapathy Pasupathy*, Latha Ravichandran, Elayaraja Sivaprakasam, Srinivasan V., Krishna Sameera G.

Department of Pediatrics, Sri Ramachandra Medical College and Research Institute, Porur, Chennai, Tamil Nadu, India

Received: 13 March 2018
Accepted: 09 April 2018

***Correspondence:**
Dr. Umapathy Pasupathy,
E-mail: drumapathy07@gmail.com

ABSTRACT

Background: Dengue is a mosquito borne viral infection caused by one of the four serotypes of dengue viruses (DENV1-DENV4). The consequences of DENV infection range from asymptomatic condition, dengue fever (DF), or severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The host immune responses have been considered as the major factor responsible for dengue pathogenesis. In this study, the cytokine IL-12 is reviewed for its utility as potential biomarker of severe dengue disease.
Methods: 120 children of paediatric age group with either dengue NS1 antigen or dengue IgM positive were included. Cases were classified as uncomplicated dengue (dengue without warning signs) and complicated dengue (dengue with warning signs and severe dengue). Clinical features and IL-12 (ELISA KIT) levels were analyzed in the study population.
Results: Analysis of clinical features among the study groups revealed children with complicated dengue had persistent vomiting (95%), abdominal pain (80%), decreased urine output (50%), bleeding manifestations (83.3%), Hepatomegaly (70%) Haemoconcentration with concurrent thrombocytopenia (93.3%), altered coagulation profile (28.3%), ICU stay (54.7%), leukocytosis (15%), leucopoenia (66.6%) normal leucocytes, (18.4%). Analysis of IL-12 levels revealed children with complicated dengue showed significant elevation compared to controls and uncomplicated dengue.
Conclusions: In our study IL-12 levels were significantly higher in complicated dengue patients in comparison with uncomplicated dengue patients as well as normal control population.

Keywords: IL-12 and dengue, Predictors of dengue severity, Clinical features and dengue, Dengue and children

INTRODUCTION

Dengue is a mosquito borne viral infection caused by one of the four serotypes of dengue viruses (Denv1-Denv4). Over 2.5 billion people of the world's population are now at risk for dengue. Dengue infections are a worldwide phenomenon spread throughout the tropical and subtropical zones.

The dengue virus is carried and introduced into the human host by the female Aedes mosquito. The global incidence of dengue has significantly increased over the past decade and is now endemic in many developing countries. South-East Asian countries such as India, Indonesia, Myanmar and Thailand are at the highest risk of dengue accounting for nearly half of the global risk.[1] Factors that have contributed to the dramatic expansion of dengue include population growth, urbanization, and inadequate water management leading to mosquito proliferation sites and convenient global travel.

The consequences of Denv infection range from asymptomatic condition, dengue fever (DF), or severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[2] Severe dengue is characterized either by plasma leakage, fluid accumulation, respiratory distress, severe bleeding or organ impairment. Clinical manifestations offer the earliest markers in predicting severe dengue disease.

A recent meta-analysis of signs and symptoms of severe dengue shows that bleeding, nausea and vomiting, abdominal pain, skin rashes, and hepatosplenomegaly are associated with severe dengue disease. Patients with dengue fever were clustered into two groups: one with warning signs including abdominal pain, mucosal bleeding and liver enlargement that warrant ICU admission and the other without those signs[3]. Early prediction of severe dengue in patients without any warning signs who may later develop severe DHF is very important to give the best supportive care since approved vaccines for immunization are yet to be commercialized. An ideal biomarker should be able to identify individuals who are at risk of developing severe dengue.

The mechanism by which only a few Denv infected individuals' progress to severe dengue disease is poorly understood. The host immune responses have been considered as the major factor responsible for dengue pathogenesis.

The process of plasma leakage, shock and hemorrhagic manifestations initiated by enhancing infection with Denv virus with the help of opsonising antibodies, resulting in an altered immune response which triggers T cell activation and release of cytokines and chemical mediators has been a risk factor in secondary infection. However, undefined factors could play a role in the development of severe dengue in individuals with primary infection and immune non-response.

Dengue patients show fever symptoms during peak of viremia while DHF/DSS appears during the time when the virus has been cleared from the circulation suggesting severe dengue disease is most likely associated with immunopathology. Thus, the host immune response components including cells, cytokines, complements and other cellular mediators can serve as biomarkers of the disease.

It is reported that the macro-morphology of endothelial lining remains intact while the functionality of the endothelial cells is altered by activation which leads to vascular permeability resulting in plasma leakage.

Therefore, endothelial activation markers such as expression of adhesion molecules and receptors can also serve as biomarkers of severe dengue disease. In present study, the cytokine IL-12 is reviewed for their utility as potential biomarker of severe dengue disease.

METHODS

Children with dengue (NS1 antigen/IgM positive) admitted as inpatients were taken as cases and afebrile children admitted for non-infectious causes in Sri Ramachandra Medical College and Research Institute were controls and were enrolled for the study.

Inclusion criteria

120 children of paediatric age group from 1 month till 18 years of age with fever for more than 5 days with either dengue NS1 antigen or dengue IgM positive were cases. 30 children who were admitted for non-infectious disease (eg.surgery) without fever and without any systemic illness and pre-existing illness (tuberculosis, asthma) in Sri Ramachandra Medical College and Research Institute were controls.

Exclusion criteria

Children with the following

- Under immunumodulatory treatment (e.g. Steroids),
- Chronic ailments (e.g. Bronchial asthma),
- Systemic illness (e.g. Malaria, Tuberculosis, HIV etc), and
- Children of parents who did not give consent for the study.

Consent was obtained and history was elicited. After physical examination and basic investigations including dengue reports, patients were categorised into 3 categories as per WHO classification of dengue, 2009[3].Controls were categorised into three groups as 1month-6years, 6years-12years, 12years – 18years irrespective of the gender. Children with coexisting illness, chronic ailments and who were on immune modulatory treatments (exclusion criteria) were not enrolled in the study.

Cases were diagnosed as dengue without warning signs, dengue with warning signs and severe dengue and were further categorised into two groups as uncomplicated dengue (dengue without warning signs) and complicated dengue (dengue with warning signs and severe dengue) and samples were collected. 120 (60+60) blood samples were collected and stored at -50^{o} centigrade. Children who were admitted for other reasons (afebrile, non-infectious causes) were divided into 3 groups as 1month to 6years, 6 years to 12 years and 12 years to 18 years and 10 samples were collected from each group.

120 case samples were collected. 60 cases were dengue without warning signs, 57 were dengue with warning signs and 3 were severe dengue. Samples were processed along with 30 controls. Samples were processed by ELISA technique.

Enzyme Linked Immuno Sorbent Assay (ELISA) is a

biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a given sample. Sandwich ELISA, which recognises both natural and recombinant human IL-12, was used. Human IL-12 ELISA kits manufactured by Diaclone were used. It contained 96 well microtitre strip plates, wash buffer, standard diluent buffer, standard controls, biotinylated anti IL-12, streptavidin-HRP. The sensitivity of IL-12 kit was 2.2pg/ml. The specificity of the kit was good that it did not show any cross reactivity when tested for any other protein. The methodology of testing was followed as given in the manual.

Statistical analysis was done using statistical package for the social sciences 17 (SPSS17) software.

RESULTS

Demographic characteristics

Children of adolescent age group were the majority and more predominated in complicated dengue (55%).

Table 1: Clinical features among complicated and uncomplicated Dengue (N=111).

		Diagnosisis		Total
		Uncomplicated dengue	Complicated dengue	
Vomiting	Yes	0	57	57
	No	60	3	63
Abdominal Pain	Yes	0	48	48
	No	60	12	72
Decreased Urine Output	Yes	1	30	31
	No	59	30	89
Mucosal Bleed	Yes	0	50	50
	No	60	10	70
Hepatomegaly	Yes	0	42	42
	No	60	18	78
Hemo concentration	Yes	0	56	56
	No	60	4	64
Altered Coagulation Profile	Yes	0	17	17
	No	60	43	103
ICU Stay	Yes	0	34	34
	No	60	26	86
WBC	Leucopoenia	39	39	78
	Leococytosis	3	9	12
	Normal	18	12	30

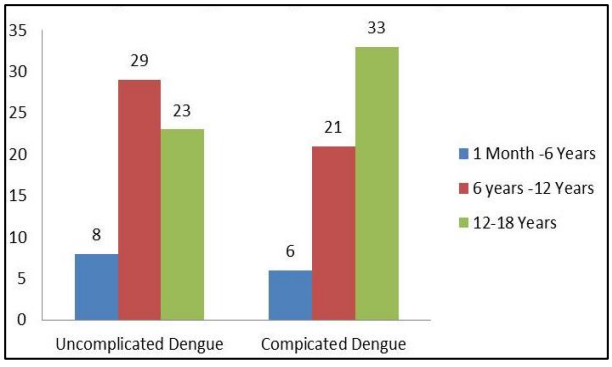

Figure 1: Age group of study group.

Almost 5% (3) children with complicated dengue did not have persistent vomiting. 20% of the children with complicated dengue did not have any significant abdominal pain while 80% of children had.

While 50% of children with complicated dengue had decreased urine output, 50% of them did not have such manifestation. 50 children (83.3%) with complicated dengue had bleeding manifestations while 16.7% did not have such episode during the illness.

Although 42(70%) children with complicated dengue had Hepatomegaly, 30% of children did not have any significant hepatomegaly. 93.3% children with complicated dengue had

Hemoconcentration with concurrent thrombocytopenia during the illness. 43 children (i.e.71.7%) with complicated dengue did not have altered coagulation profile while 17 children from the same group had altered coagulation.

When none of the patients with uncomplicated dengue required ICU stay, 26(43.3%) children with complicated

dengue also did not require any ICU management. Out of 60 children with complicated dengue, 9 (15%) had leucocytosis and 39 (66.6%) had leucopoenia while rest of the children 12(18.4%) had normal leucocytes.

Descriptive statistics

This study was done among dengue patients where 60 complicated cases of dengue and 60 uncomplicated cases of dengue were taken along with 30 healthy controls.

Other characteristics like demographic details, clinical features, and blood investigations were also taken into account. All the study participants were analysed for IL-12.

Table 2: Descriptive statistics.

	N	Minimum	Maximum	Mean	Std. deviation
IL 12	150	0.00	2000	561.4062	260.47321

Table 1 shows the mean level for IL-12 in study population 561.40 and the standard deviation was 560.47. Subjects analysed simultaneously with statistical test of significance by applying ANOVA (Analysis of the Variance) between the groups and within the groups where between the group was complicated vs uncomplicated dengue whereas within the group indicates complicated / uncomplicated vs normal healthy controls.

Table 3: ANOVA.

	Sum of squares	DF	Mean square	F	SIG.
IL 12 between groups	8401199	2	4200599	15.079	0.000
within groups	38404204	147	251253		
total	45805403	149			

It has been observed from Table 2 that the mean square difference was higher between the groups when compared within the group.

The difference of observation was found statistically significant in IL-12 (p=0.000) by using F test. F test value was 16.079 times protective when compared to mean square difference.The 95% Confidence Interval of the mean ranges from 588.59 to 847.48 in relation to IL-12. The higher formation of mean value was found to be high with respect to IL-12.

Table 4: Descriptives.

	N	Mean	Std. dev	95% Ci for mean		Std error	Minimum	Maximum
				Lower bound	Upper bound			
Uncomplicated dengue	60	703.0385	559.16378	558.5912	847.4858	72.18773	23.15	2000
Complicated dengue	60	655.4762	577.48644	506.2956	804.6567	74.55318	0.00	2000
Normal	30	90.0017	98.44926	53.2401	126.7632	17.97429	0.00	388.95

Table 5: Multiple comparisons.

Diagnosis (I)	Diagnosis (J)	Mean Diff (I – J)	Std Error	Significance	95% Conf Int	
					Lower Bound	Upper Bound
Un complicated Dengue	Complicated dengue	47.56233	93.319	0.867	-0.173.3884	268.5131
	Normal	613.03683	114.29197	0.000	342.4285	883.6452
Complicated Dengue	Uncomplicated dengue	-47.56233	93.319	0.867	-268.5131	173.3884
	Normal	565.4745	114.29197	0.000	294.8662	836.0828
Normal	Uncomplicated dengue	-613.03683	114.29197	0.000	-883.6452	-342.4285
	Normal	-565.47450	114.29197	0.000	-836.0828	-294.8662

Analysis of the study using test of significance.

Mean IL-12 levels in both groups (complicated and uncomplicated dengue) were found to be significantly elevated (p value 0.000) when compared with normal healthy controls. But it was observed that mean IL-12 levels in uncomplicated dengue group was higher than complicated dengue, which may suggest the role of IL-12 in Th1 pathway mediated protective response seen in

uncomplicated dengue. However, the difference was statistically insignificant.

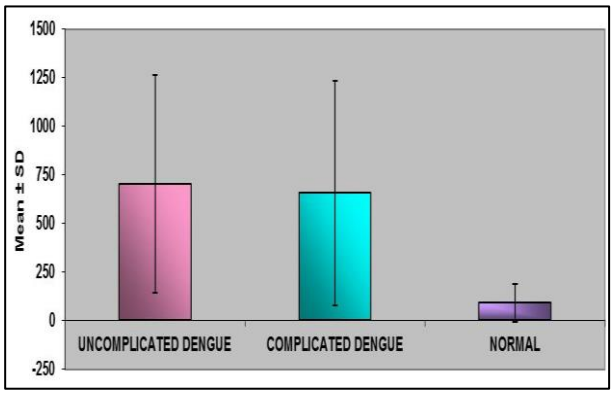

Figure 2: IL-12.

ROC Curve

This test was done to find out the reliability of IL-12 for application in dengue by using recessive operative characteristics (ROC) curve. Figure 6 depicts ROC curve for IL-12.

Table 6: Area under the curve.

Area	Std. Error	Asymptotic Sig	Asymptomatic 95% confidence interval	
			Lower bound	Upper bound
895	0.027	0.000	0.843	0.948

Figure 6 shows that the area under the ROC curve for IL-12 is 0.895 (89%) which indicate good sensitivity and specificity of the test in diagnosing severity of dengue. Hence it can be observed that the reliability of IL-12 is high in evaluating dengue patients.

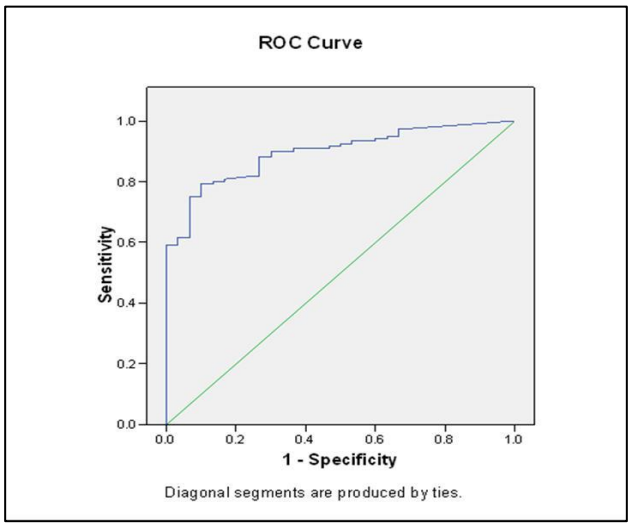

Figure 3: ROC curve of IL-12.

DISCUSSION

The present study aimed to determine the significance of cytokine IL-12 in children with dengue, dengue with warning signs and severe dengue in comparison with normal children as controls. In addition, the study also aims to determine IL-12 as predictor of dengue severity. In our study, 120 children with dengue were enrolled as cases along with 30 controls. The 120 children in the case group had 60 children with complicated dengue and 60 children with uncomplicated dengue. The normal healthy controls were divided into 3 groups as 1 month- 6 years, 6 years-12 years and 12 years-18 years. Previously, few studies have reported the incidence of 50 million dengue cases per year occurring worldwide that includes more than 5,00,000 cases of severe dengue.[4-5]

Why IL-12 was analysed?

In our study, a cytokine from the Th1 pathway, which shows a significant variation during 5-8 days of illness were analysed. In a similar study by Chaturvedi et al similar analysis was carried out along with other cytokines like IL-5, IL-6, and IL-10 etc.[6]

Similarly, IL-12 is a key cytokine in the Th1 response and therefore has a protective effect with elevated IL-12 levels seen in uncomplicated dengue. Thus IL-12 elevation has been observed in uncomplicated dengue patients. The other interesting feature is significant variation in levels of cytokine IL-12) were observed only during day 5-day 8 of illness. Hence, we studied the level of IL-12 in dengue patients between 5-8 days of illness.

Kumar Y et al, studied 27 cytokines levels in 62 adult patients, showed elevated levels of IL-12.[7] IL-12 levels were similarly elevated in our study when comparing dengue children with healthy control.

In a study by Braiser et al, where 55 individuals with dengue were analysed for various cytokines showed significant difference only in levels of IL-6 and IL-10 whereas in present study IL-12 were significant when compared between milder and severe dengue and IL-12 was significant when it was compared with healthy controls.[8]

A case control study by Sing et al, involving 120 controls and 86 DF patients and 196 patients with DHF/DSS were analysed which showed IL-12 has a protective role against development of severe dengue in patients with milder dengue.[9] Although in our study, the levels of IL-12 was low in complicated dengue when compared to children with milder dengue, it was not statistically significant (p value was 0.867).

In line with our study, a case reported by Masaki et al, a Japanese traveller infected by dengue virus serotype 3 showed elevated levels of IL-12 in dengue fever.[10] The study also showed a marked decrease in CD4 cells and

significant increase in Interferron gamma in DF. In a study involving 66 paediatric dengue patients to establish the relation between soluble ST2 and IL-33 in severe dengue, the results showed higher levels of IL-12 in children with dengue fever. This study done by Guerrero et al, showed similar results with regarding IL-12.[11] Majority of the international and national studies showed an elevation of Th1 mediated cytokine response in the acute phase of dengue infection. Few studies have also demonstrated the predictive role of IL-12 (Th2 mediated cytokine) in non severe dengue patients. Our study was in concordance with the findings of these studies.

Limitations of present study are:

- Relatively low sample size in present study.
- Present study was restricted to a single urban centre where the exact magnitude of the prevailing disease may not be similar.
- No clear cut reference ranges for IL-12 were available and hence control values were taken for reference ranges.
- Values of IL-12 had a wide range of variation. Negative values from ELISA reader were approximated to 0 as minimum.

CONCLUSION

In present study IL-12, the levels are elevated in both complicated and uncomplicated dengue; it is relatively lower in complicated dengue than in uncomplicated dengue children. Hence, IL-12 elevation has a protective role in the evolution of dengue illness and prevents serious forms of illness.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

REFERENCES

1. WHO/SEARO. Concrete measure key in controlling dengue in South East Asia. Press Release SEA/PR/1479. New Delhi, World Health Organization Regional Office for South-East Asia, 2008. Available at http://www.searo.who.int/EN/Section316/Section503/Section2463_14619.htm.
2. WHO. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control, 2nd ed. Geneva, World Health Organization, 1997.
3. World Health Organization. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control. Geneva, Switzerland: WHO, 2009.
4. Halstead SB. Dengue. Lancet (London, England). 2007;370(9599):1644-52.
5. Chen LC, Lei HY, Liu CC. Correlation of serum levels of macrophage migration inhibitory factor with disease severity and clinical outcome in dengue patients. Am J Trop Med Hyg. 2006;74(1):142-7.
6. Chaturvedi UC, Agarwal R, Elbishbishi EA, Mustafa AS. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol Med Microbiol. 2000;28(3):183-8.
7. Kumar Y, Liang C, Bo Z, Rajapakse JC, Ooi EE, Tannenbaum SR. Serum proteome and cytokine analysis in a longitudinal cohort of adults with primary dengue infection reveals predictive markers of DHF. PLoS Negl Trop Dis. 2012;6(11):e1887.
8. Brasier AR, Garcia J, Wiktorowicz JE, Spratt HM, Comach G, Ju H, Recinos A et al. Discovery proteomics and nonparametric modeling pipeline in the development of a candidate biomarker panel for dengue hemorrhagic fever. Clin Transl Sci. 2012 February;5(1):8-20.
9. Sam SS, Teoh BT, Chinna K, AbuBakar S.. High producing tumor necrosis factor alpha gene alleles in protection against severe manifestations of dengue. Int J Med Sci. 2015;12(2):177-186.
10. Masaki H, Hasebe F, Ahmed K, Fukuda T, Furumoto A, Watanabe K, Oishi K, Igarashi A, Nagatake T. A clinical, serological, and immunological study in a Japanese traveler with dengue fever. J Infect Chemother. 2002 Dec; 8(4):365-7.
11. Guerrero CD, Arrieta AF, Ramirez ND, Rodríguez LS, Vega R, Bosch I et al. High plasma levels of soluble ST2 but not its ligand IL-33 is associated with severe forms of pediatric dengue "an official journal of the international cytokine and interferon society. 2013;61(3):766-71.

Cite this article as: Manoharan R, Pasupathy U, Ravichandran L, Sivaprakasam, E Srinivasan V., Sameera KG. Cytokine IL-12, Dengue in children: analysis of a unique relationship. Int J Contemp Pediatr 2018;5:1109-14.