CLINICAL FEATURES AND SIGNIFICANCE OF CYTOKINE IL-4 IN CHILDREN WITH DENGUE AT A TERTIARY CARE CENTRE

Rakesh Manoharan[1], Umapathy Pasupathy[2], Elayaraja Sivaprakasam[3], Latha Ravichandran[4], Srinivasan Venkataraman[5], Dhana Rathna Moorthy Vetrichelvan[6]

[1]Postgraduate Student, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.
[2]Associate Professor, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.
[3]Assistant Professor, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.
[4]Professor, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.
[5]Student, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.
[6]Postgraduate Student, Department of Paediatrics, Sri Ramachandra Medical College and Research Institute, Chennai.

ABSTRACT

BACKGROUND

Dengue is a mosquito borne viral infection in tropical and subtropical regions 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 Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). The host immune responses have been considered as the major factor responsible for dengue pathogenesis. Endothelial activation markers such as expression of adhesion molecules and receptors have been found to serve as biomarkers of severe dengue disease. In this study, the cytokine IL-4 is reviewed for its utility as potential biomarker of severe dengue disease.

MATERIALS AND METHODS

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 included. 30 children who were admitted for noninfectious disease (e.g. surgery) without fever, any systemic illness and preexisting illness (tuberculosis, asthma) in SRMC and RI were taken as controls. Cases were classified as uncomplicated dengue (dengue without warning signs) and complicated dengue (dengue with warning signs and severe dengue). Clinical features and IL-4 (ELISA kit) were analysed and compared among the study population and statistical analysis done for the obtained data.

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%), leucocytosis (15%), leucopenia (66.6%) and normal leucocytes (18.4%). Analysis of IL-4 levels revealed children with complicated dengue showed >6 fold elevation in IL-4 levels (p=0.003). Mean IL-4 levels in complicated dengue group was also statistically significant (p=0.013). ROC curve for IL-4 indicated 50% reliability as predictor for dengue severity.

CONCLUSION

In our study, IL-4 levels were significantly higher in complicated dengue patients in comparison with uncomplicated dengue patients as well as normal control population. Though, this interleukin 4 can be used for assessing the severity of children with dengue, further studies with higher sample size are needed to advocate the routine use of IL-4 as a biomarker of dengue severity.

KEYWORDS

IL-4 and Dengue, Predictors of Dengue Severity, Clinical Features and Dengue, Dengue and Children.

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Corresponding Author:
Dr. Umapathy Pasupathy,
Plot No. 149, Mangala Nagar,
5th Cross Street, Porur,
Chennai-600116, Tamil Nadu.
E-mail: drumapathy07@gmail.com
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BACKGROUND

Dengue is a mosquito borne viral infection found in tropical and subtropical regions of the world and is caused by one of the four serotypes of dengue viruses (DENV1-DENV4), which is carried and introduced into human host by the female Aedes mosquito. An increase in infection has been seen in recent years due to many factors including urbanisation and air travel. Over 2.5 billion people of the world's population are now at risk for dengue.

The global incidence of dengue has significantly increased over the past decade and is now endemic in many developing countries. South East Asian countries 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, urbanisation 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 Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).[2] Severe dengue is characterised 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 immunisation are yet to be commercialised. 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. Dengue patients show fever symptoms during peak of viraemia 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 immune pathology.[4] 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 macromorphology 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.[4] Therefore, endothelial activation markers such as expression of adhesion molecules and receptors can also serve as biomarkers of severe dengue disease. In our study, the cytokine IL-4 is reviewed for its utility as potential biomarker of severe dengue disease.

MATERIALS AND METHODS

Children with dengue (NS1 antigen/IgM positive) admitted as inpatients were taken as cases and afebrile children admitted for noninfectious causes in Sri Ramachandra Medical College and Research Institute were taken as controls 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 included. 30 children who were admitted for noninfectious disease (e.g. surgery) without fever in Sri Ramachandra Medical College and Research Institute were taken as controls.

Exclusion Criteria

Children who were-

1. Under immunomodulatory treatment (e.g. steroids).
2. Chronic ailments (e.g. bronchial asthma).
3. Systemic illness (e.g. malaria, tuberculosis, HIV, etc.).
4. Children of parents who did not give consent for the study.

Informed consent was obtained and history was elicited. Based on physical examination and basic investigations, patients were categorised into 3 categories as per WHO classification of dengue, 20093. Controls were categorised into three groups as 1 month to 6 years, 6 years to 12 years, 12 years to 18 years irrespective of the gender.

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° centigrade. Children who were admitted for other reasons (afebrile, noninfectious causes) were divided into 3 groups as 1 month to 6 years, 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 Immunosorbent 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-4 was used. Human IL-4 ELISA kit manufactured by Diaclone was used. It contained 96 well microtiter strip plates, wash buffer, standard diluent buffer, standard controls, biotinylated anti IL-4, streptavidin-HRP. The sensitivity of IL-4 kit was 0.7 pg/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 for each was followed as given in the manual. The data collected was tabulated and results were analysed. Statistical analysis was done using statistical package for the social sciences 17 (SPSS17) software.

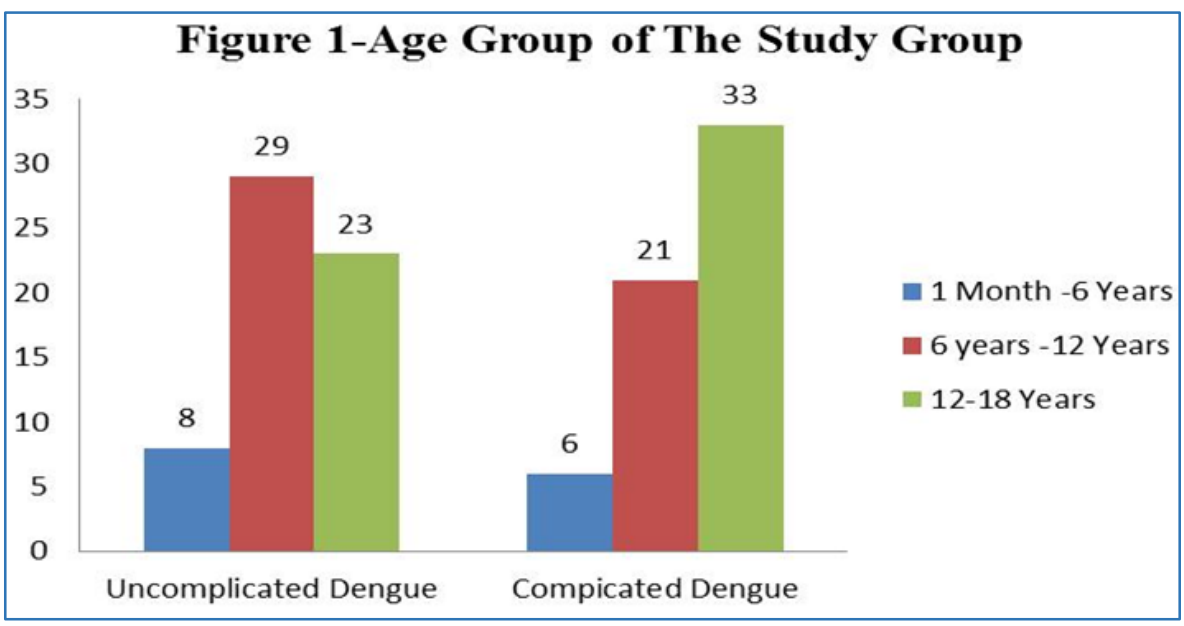
OBSERVATION AND RESULTS

Demographic Characteristics

			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Age	1 month-6 years	Count	8	6	14
		% Within Diagnosis	13.3	10	11.7
	6 years-12 years	Count	29	21	50
		% Within Diagnosis	48.3	35	41.7
	12 years-18 years	Count	23	33	56
		% Within Diagnosis	38.3	55	46.7
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 1. Age Group of The Population

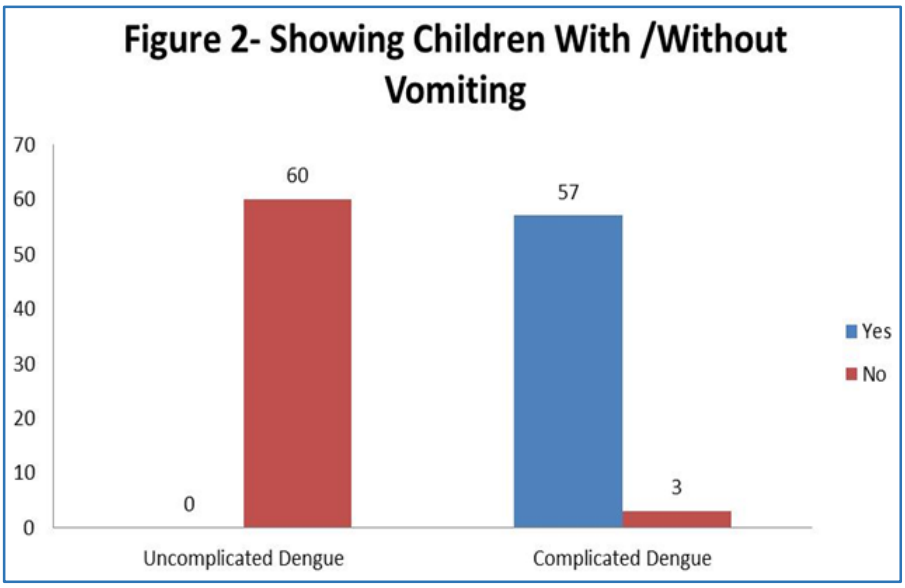
Children of adolescent age group were the majority and more predominated in complicated dengue (55%).



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Vomiting	Yes	Count	0	57	57
		% Within Diagnosis	0	95	47.5
	No	Count	60	3	63
		% Within Diagnosis	100	5	52.3
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 2. Children With/Without Vomiting

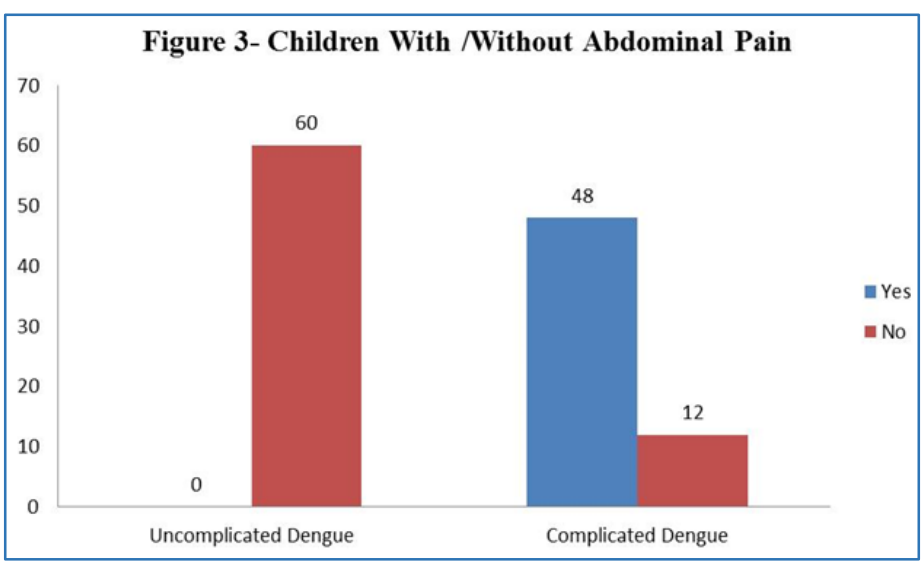
+ almost 5% (3) children with complicated dengue did not have persistent vomiting.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Abdominal Pain	Yes	Count	0	48	48
		% Within Diagnosis	0	80	40
	No	Count	60	12	72
		% Within Diagnosis	100	20	60
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 3. Children With/Without Abdominal Pain

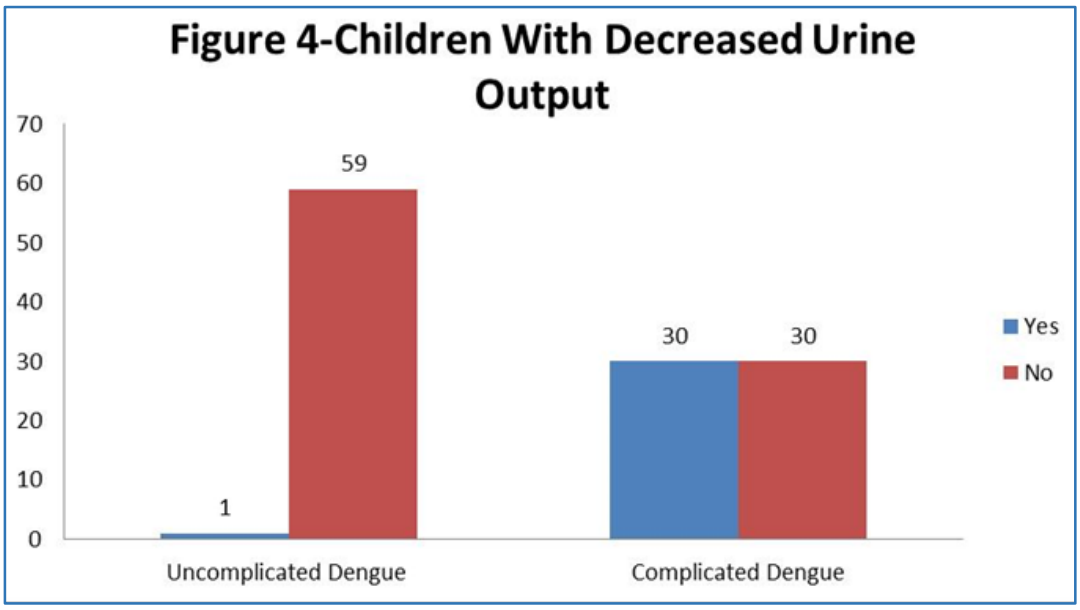
- 20% of the children with complicated dengue did not have any significant abdominal pain while 80% of the children had.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Decreased Urine	Yes	Count	1	30	31
		% Within Diagnosis	1.7	50	25.8
	No	Count	59	30	89
		% Within Diagnosis	98.3	50	74.2
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 4. Children With/Without Decreased Urine Output

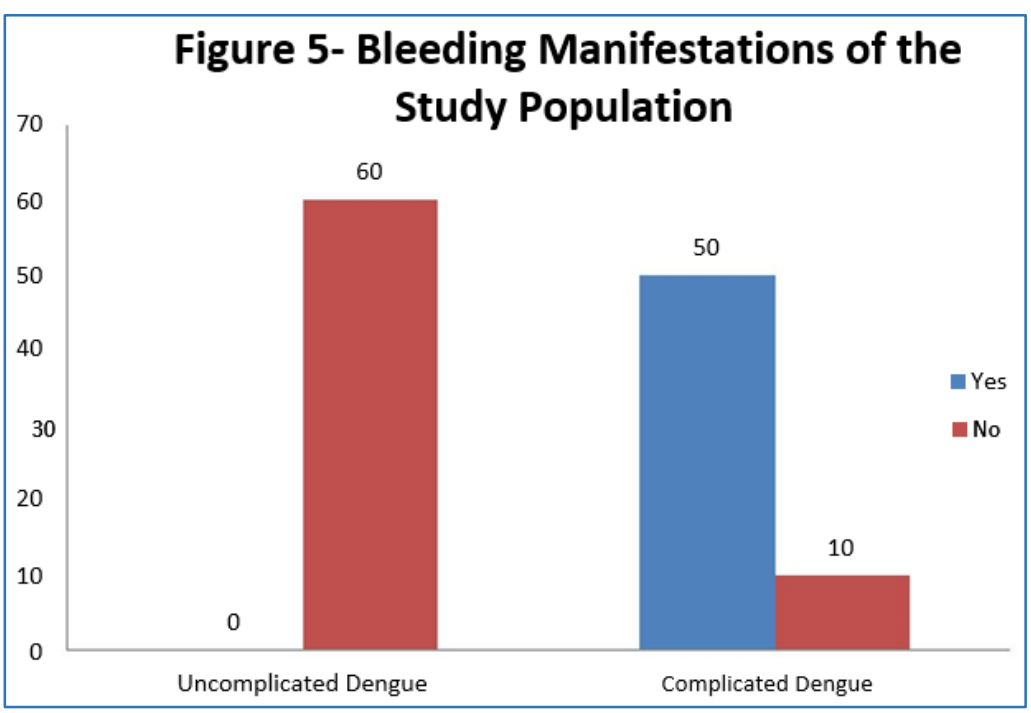
- While 50% of the children with complicated dengue had decreased urine output, 50% of them did not have such manifestation.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Mucosal Bleed	Yes	Count	0	50	50
		% Within Diagnosis	0	83.5	41.7
	No	Count	60	10	70
		% Within Diagnosis	100	16.7	58.3
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 5. Mucosal Bleed of The Study Population

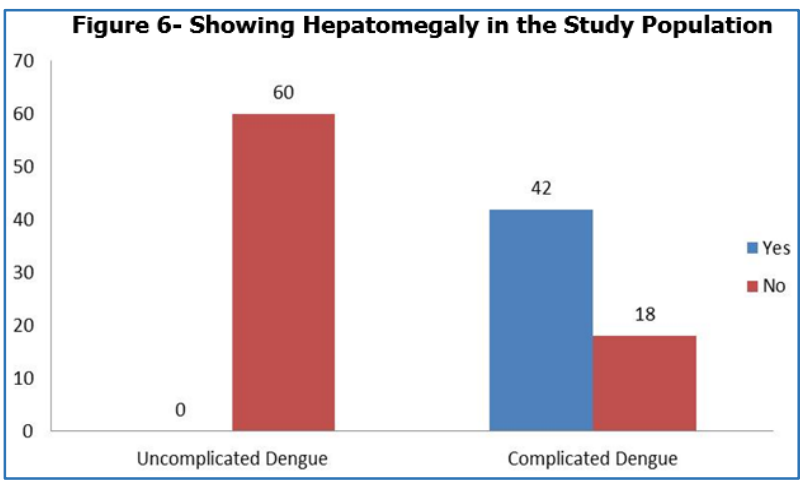
- 50 children (83.3%) with complicated dengue had bleeding manifestations while 16.7% did not have such episode during the illness.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Hepatomegaly	Yes	Count	0	42	42
		% Within Diagnosis	0	70	35
	No	Count	60	18	78
		% Within Diagnosis	100	30	65
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 6. Hepatomegaly of the Study Population

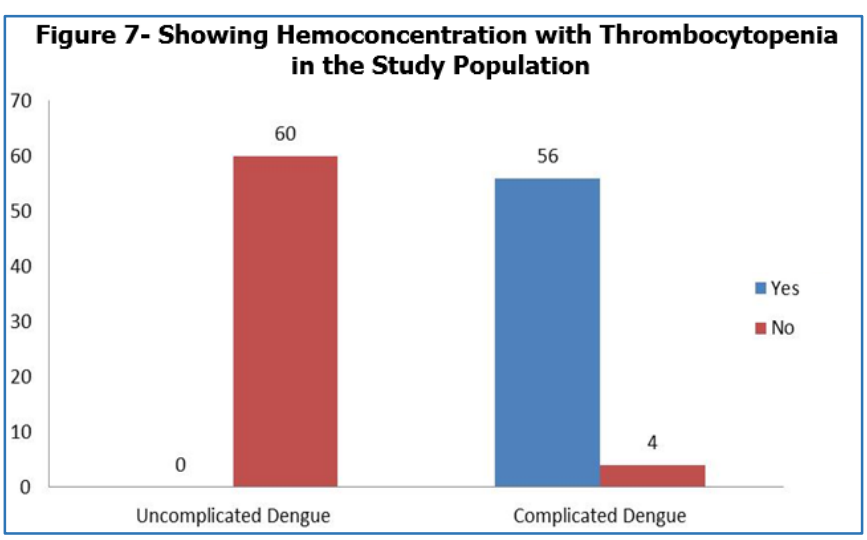
- Although, 42 (70%) of the children with complicated dengue had hepatomegaly, 30% did not have any significant hepatomegaly.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Haemoconcentration	Yes	Count	0	56	56
		% Within Diagnosis	0	93.3	46.7
	No	Count	60	4	64
		% Within Diagnosis	100	6.7	53.3
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 7. Haemoconcentration With Thrombocytopenia of the Study Population

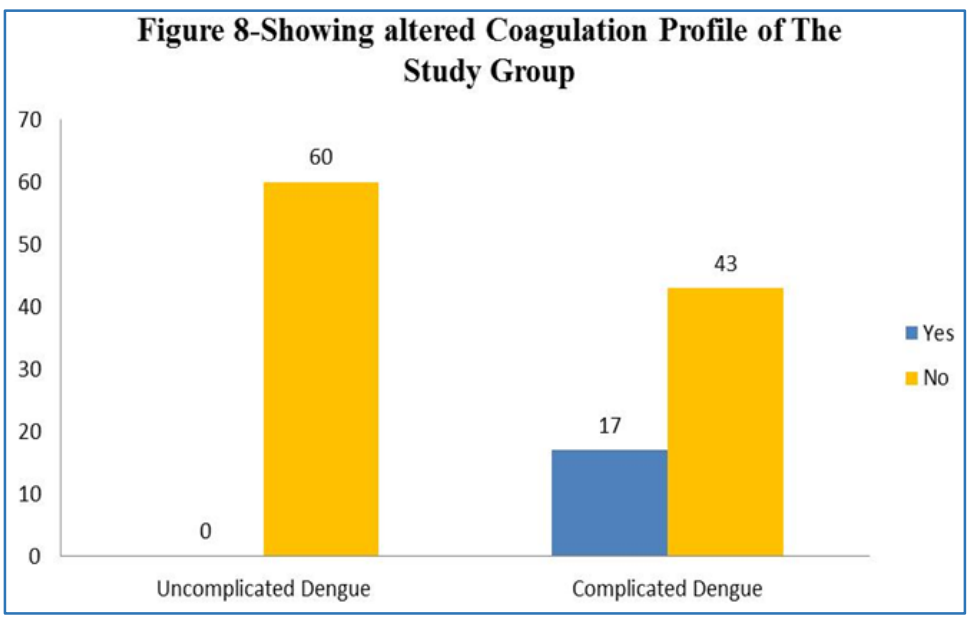
- 93.3% children with complicated dengue had haemoconcentration with concurrent thrombocytopenia during the illness.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
Altered Coagulation Profile	Yes	Count	0	17	17
		% Within Diagnosis	0	26.3	14.2
	No	Count	60	43	103
		% Within Diagnosis	100	71.7	85.8
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 8. Altered Coagulation Profile of the Study Group

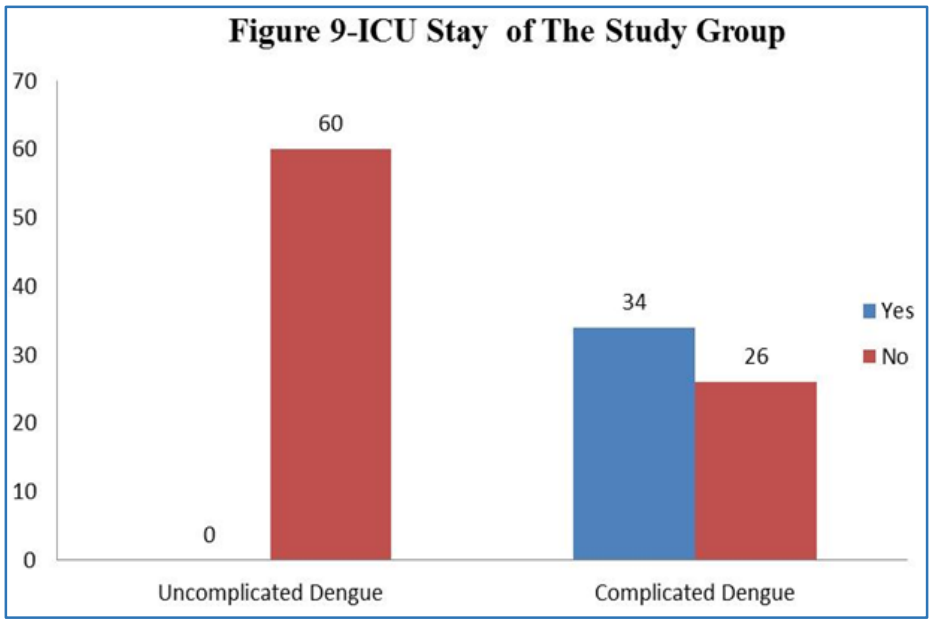
- 43 children (71.7%) with complicated dengue did not have altered coagulation profile while 17 children from the same group had altered coagulation.



			Diagnosis		Total
			Uncomplicated Dengue	Complicated Dengue	
ICU Stay	Yes	Count	0	34	34
		% Within Diagnosis	0	56.7	28.3
	No	Count	60	26	86
		% Within Diagnosis	100	43.3	71.7
Total		**Count**	**60**	**60**	**120**
		% Within Diagnosis	**100**	**100**	**100**

Table 9. ICU Stay of The Study Group

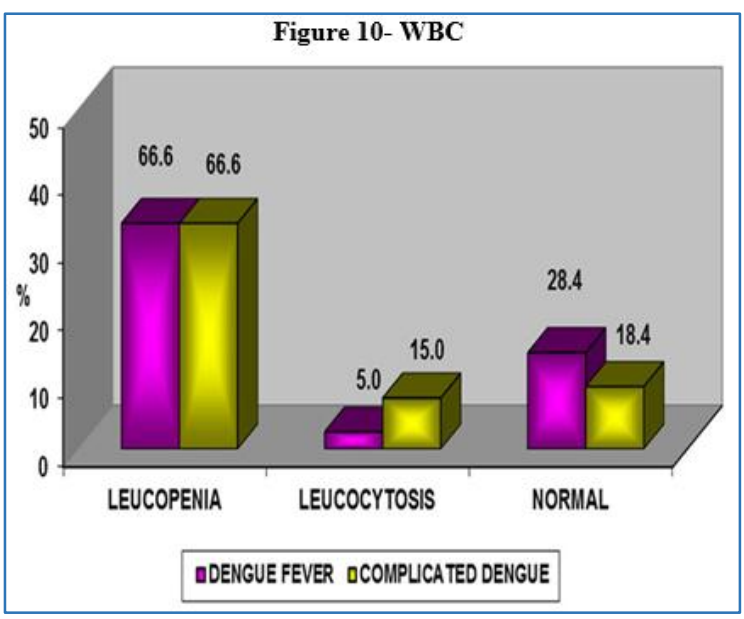
- When none of the patients with uncomplicated dengue require ICU stay, 26 (43.3%) children with complicated dengue also did not require any ICU management.



WBC	Leukopenia	Leukocytosis	Normal	Total
Uncomplicated Dengue	66.6%	5%	28.4%	100%
Complicated Dengue	66.6%	15%	18.4%	100%
Total	**133.2%**	**20%**	**46.8%**	**200%**

Table 10. WBC of the Study Population

- Out of 60 children with complicated dengue, 9 (15%) had leukocytosis and 39 (66.6%) had leukopenia while rest of the children 12 (18.4%) had normal leukocytes.



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-4.

	N	Minimum	Maximum	Mean	Std. Deviation
IL4	150	0.00	14.50	0.5807	1.95152
Valid N (list wise)	150				

Table 11. Descriptive Statistics

Table-11 shows the mean level of IL-4 in study population was 0.6807 and the standard deviation was 1.95, respectively. 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.

IL 4	Sum of Squares	DF	Mean Square	F	Sig.
Between Groups	49.540	2	24.820	7.045	0.001
Within Groups	517.816	147	3.523		
Total	**567.456**	149			

Table 12. ANOVA

It has been observed from Table 12 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-4 (p=0.001) by using F test. The F test value was 7.046 times protective when compared to mean square difference.

Analysis of Study Using Test of Significance

IL-4	N	Mean	Std. Deviation	95% CI for Mean		Std. Error	Minimum	Maximum
				Lower Bound	Upper Bound			
Uncomplicated Dengue	60	0.2287	1.37722	-0.1271	0.5844	0.17780	0.00	10.46
Complicated Dengue	60	1.3848	2.61814	0.7085	2.0612	0.33800	0.00	14.6
Normal	30	0.1763	0.22619	0.0919	0.2608	0.04130	0.00	0.65
Total	**150**	**0.6807**	**1.95162**	**0.3658**	**0.9955**	**0.15934**	**0.00**	**14.6**

Table 13. Descriptives

The 95% confidence interval of the mean ranges from -0.127 to 0.584 in relation to IL-4.

Dependent Variable	Diagnosis (I)	Diagnosis (J)	Mean Diff. (I-J)	Std. Error	Significance	95% Conf. Int.	
						Lower Bound	Upper Bound
IL-4	Uncomplicated Dengue	Complicated Dengue	-1.15617	0.34266	0.003	-1.9675	-0.3448
		Normal	0.05233	0.41968	0.991	-0.9413	1.0460
	Complicated Dengue	Uncomplicated Dengue	1.15617	0.34266	0.003	0.3448	1.9675
		Normal	1.20850	0.41968	0.013	0.2148	2.2022
	Normal	Uncomplicated Dengue	0.05233	0.41868	0.991	-1.046	0.9413
		Normal	-1.20850	0.41968	0.013	-2.2022	-0.2148

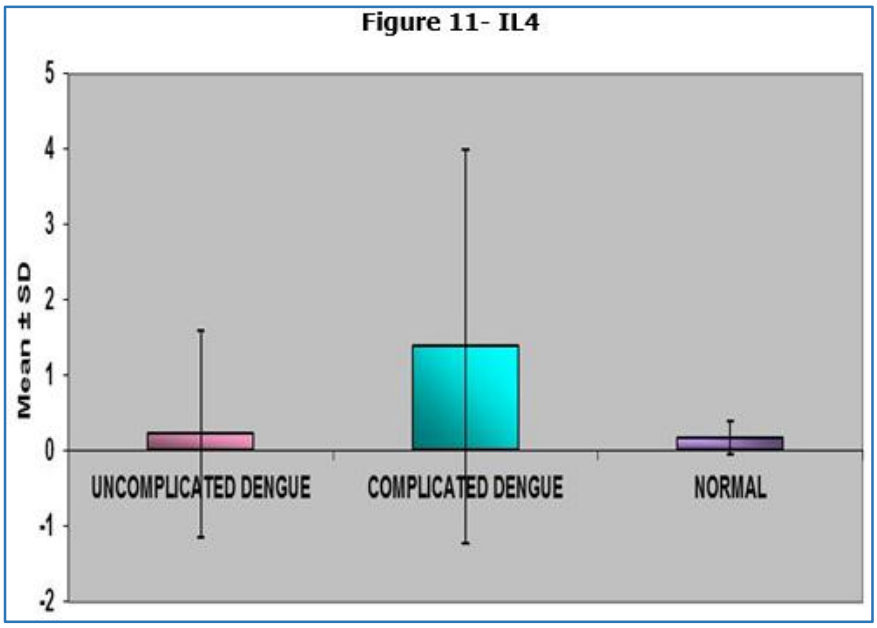
Table 14. Multiple Comparisons

Mean elevation in IL-4 levels was observed in both uncomplicated and complicated dengue groups. But, the complicated dengue group showed more than 6 fold elevation in IL-4 levels, which was statistically significant (p=0.003).

Difference in mean IL-4 levels between normal healthy controls and complicated dengue group was also statistically significant (p=0.013).

ROC Curve

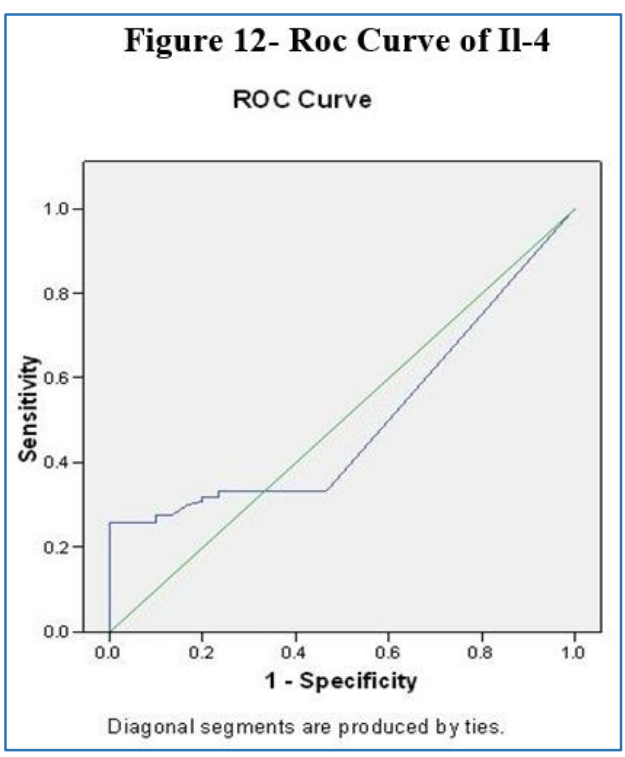
This test was done to find out the reliability of interleukin (IL-4) for application in dengue by using Recessive Operative Characteristics (ROC) curve. Figure 12 depicts ROC curve for IL-4.



Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.499	0.053	0.981	0.395	0.603

Table 15. Area Under The Curve Test Result Variable (S)- IL-4

Figure 12 shows that the area under the ROC curve for IL-4 is 0.499 indicating 50% reliability in evaluating severity of dengue.



DISCUSSION

The present study aimed to determine the significance of cytokine IL-4 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-4 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 to 6 years, 6 years to 12 years and 12 years to 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-4 was Analysed?

In our study, a cytokine from Th2 pathway, which shows a significant variation during 5-8 days of illness was analysed. In a similar study by Chaturvedi et al,[6] similar analysis was carried out along with other cytokines like IL-5, IL-6 and IL-10, etc. Also, various studies have shown that elevated IL-4 levels (a key cytokine Th2 pathway) were observed in patients with complicated dengue and hence could be used a potential biomarker for assessing the severity.

Thus, IL-4 level elevation predicted severity of dengue. The other interesting feature is significant variation in levels of cytokine (IL-4) was observed only during day 5 to day 8 of illness. Hence, we studied the levels of IL-4 in dengue patients between 5-8 days of illness.

In a prospective study by Bozza et al[7] involving 59 dengue patients the levels of IL-4, IL-6, IL-7, IL-13, IL-1β, IFN Gamma and GM-CSF were significantly elevated in severe dengue patients when compared to milder dengue (DF). This was in concordance with our study where the levels of IL-4 were significantly elevated in children with complicated dengue when compared to uncomplicated dengue. In a study done by Green S et al[8] and Pinto et al[9]

where the levels of IL-4, IL-6, IL-10 and TNF alpha of dengue children were compared with normal patients as controls, the levels of IL-4 was not significantly elevated in dengue children when compared to normal children, which was against our study.

Kumar Y et al[10] studied 27 cytokines levels in 62 adult patients showed elevated levels of IL-4 and IL-12 of which IL-4 was 10 times more elevated when compared with healthy control. This was incongruous with our study where the levels of IL-4 were not much elevated in dengue children when compared with normal children.

A prospective study done by I.K Lee et al[11] where 34 adults with type 2 diabetes (DM) with dengue was analysed showed elevated levels of IL-4, IL-10 and GM-CSF when compared with normal individuals. This was also in concordance with our results where IL-4 was elevated in children with dengue when compared to normal.

In a study by Braiser et al[12] where 55 individuals with dengue were analysed for various cytokines showed significant difference only in levels of IL-6 and IL-10, whereas in our study, IL-4 was significant when compared between milder and severe dengue.

In line with our study, Kadhiravan et al[13] showed that the levels of IL-4 was significantly elevated in patients with DHF when compared to milder dengue. This study involved 20 dengue patients and 10 healthy controls.

Ulaganathan Mabalirajan et al[14] demonstrated the decreasing trend of IL-4 in dengue patients during the defervescence phase. The study was carried out in 28 dengue patients with 60 normal as controls. This was in concordance with our study where significant number of children in our study with non-severe dengue had lower levels of IL-4.

In a prospective study done at Thailand done by Anuja Mathew et al[15] to determine the change in cell-mediated response in children during acute dengue infection showed higher levels of IL-4 during febrile phase and decreased levels during defervescence phase. In our study, the levels of IL-4 was much lower in those children with uncomplicated dengue and the samples were taken during 5-8 days of illness.

Butthep et al[16] did a detailed study in 164 paediatric patients who were admitted with DF, DHF and DSS and interleukin levels were screened during the various phase of the illness from febrile phase and till convalescent phase of the illness. He observed that the levels of IL-4, IL-6, IL-10 and TNFα were significantly high during the febrile phase of the illness. This was similar to our study results where the patients with uncomplicated dengue tend to have increased levels of IL-4. His study also stated that chemokine kinetics can be used as a predictor of severe infection.

Cytokines levels were analysed from peripheral blood mononuclear cells by Susan J Gagnon et al.[17] His results showed a greater expression of TNFα and IL-4 in DHF than in DF, which was similar to our results. Analysis of cytokines in paediatric patients affected with DENV-2 and plasmodium falciparum by Maneekan et al[18] showed no significant variations of IL-4 when compared to normal healthy controls. This was in agreement to our results.

Similarly, elevated levels of IL-4 was observed in dengue patients when compared to normal healthy controls. The healthy controls were the household member of each dengue patient and there was significant increase in IL-4 levels in dengue patients. This study was done by Yeo et al[19] involving 29 pairs in the study.

Cytokine study in dengue patients with coexisting hepatitis B (HBV) virus infection was carried out in China by Tang Y et al.[20] 353 patients were enrolled in the study and were divided into two groups (only dengue and dengue with coexisting chronic HBV infection). Dengue patients with HBV coinfection had low levels of IL-6 and interferon gamma while similar levels of IL-4 was noted in both groups.

CONCLUSION

In our study, IL-4 levels were significantly higher in complicated dengue patients in comparison with uncomplicated dengue patients as well as normal control population. Though, this interleukin 4 can be used for assessing the severity of children with dengue, further studies with higher sample size are needed to advocate the routine use of IL-4 as a biomarker of dengue severity.

LIMITATIONS OF THE STUDY

1. Relatively low sample size in our study.
2. Our study was restricted to a single urban centre where the exact magnitude of the prevailing disease may not be similar.
3. No clear-cut reference ranges for IL-4 was available and hence control values were taken for reference ranges.
4. Values of IL-4 had a wide range of variation. ELISA reader did not show any reading for negative values, which were approximated to 0 as minimum and it was noted in 96 samples for IL-4. Due to this reason, there was a large variation of SD was found in IL-4 values.

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