
**“IL-6 LEVELS IN PREDICTION OF SEVERITY OF
DENGUE FEVER – CROSS SECTIONAL
OBSERVATIONAL STUDY”**

BY

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LIST OF ABBREVIATIONS

AD	-	Assay Diluent
DF	-	Dengue Fever
DIC	-	Disseminated Intravascular Coagulation
DHF	-	Dengue Haemorrhagic Fever
DSS	-	Dengue Shock Syndrome
DV	-	Dengue Virus
ELISA	-	Enzyme Linked Immunosorbent Assay
HUVEC	-	Human Umbilical Vein Endothelial Cells
IFN	-	Interferon
IL	-	Interleukin
NS1	-	Non-Structural Protein 1
NPV	-	Negative Predictive Value
PAF	-	Platelet Activating Factor
PICU	-	Paediatric Intensive Care Unit
PPV	-	Positive Predictive Value
ROC	-	Receiver Operating Characteristic Curve
RT-PCR	-	Reverse Transcription Polymerase Chain Reaction
SD	-	Standard Deviation
TNF	-	Tumour Necrosis Factor
tPA	-	Tissue Plasminogen Activator
WHO	-	World Health Organisation

ABSTRACT

INTRODUCTION-

Interleukin-6 (IL-6) is an extremely varied immunomodulatory cytokine which is associated with the generation of anti platelet, anti endothelial autoantibody, and coagulation defects leading to bleeding and plasma leakage in DV infection and causing severe dengue fever.

AIMS AND OBJECTIVES-

PRIMARY

IL-6 levels in dengue fever to predict severity

SECONDARY

To correlate IL-6 levels with platelet count in patients with dengue fever.

MATERIAL AND METHODS-

One year hospital based cross-sectional study was conducted with sample size of 60 patients who met the inclusion criteria i.e. all were dengue NS1 or IgG or IgM positive. The serum samples were collected on day 1 and 4 of admission at KLE hospital, Belgaum and centrifuged at 3600 rpm and stored at -80°C. The samples were then subjected to 1:3 dilution and then IL-6 levels were checked in doublets by ELISA kits of Krishgen Biosystems. Data was analysed using statistical software R version 4.1.2. To check the normality of variables Shapiro-Wilk's test and box-plot is used. P-value less than or equal to 0.05 indicates statistical significance. To find the cut point of IL-6 levels at day1 and day4 with respect to severity of dengue and outcome(expired) diagnostic test is performed and sensitivity, specificity, NPV and PPV were reported.

RESULTS-

p-value of 0.0176 and 0.0025 on day 1 and 4 of admission was obtained respectively comparing IL-6 levels with severity of dengue fever. While correlating IL-6 levels on day 1 with the platelet counts on day 4 p-value of 0.0070 was obtained, similarly p value of 0.0034 and 0.0391 was obtained correlating IL-6 values on day 4 with platelet counts on day 3 and 4 respectively. By spearman correlation there exists a low negative correlation between **IL-6 levels at day1** and platelet count from day 2 to day7 and low positive correlation between **IL-6 levels at day1** and platelet count at day1. Similarly there exists a low negative correlation between IL-6 levels at day1 and platelet count from day 2 to day7 and low positive correlation between IL-6 levels at day1 and platelet count at day1.

CONCLUSION-

IL-6 levels correlate with severity of dengue fever, higher values on day 1 and 4 signifies severe dengue similarly lower values signifies non- severe dengue. Also, IL-6 values correlate with the platelet count as they show negative correlation with platelet count which means if IL-6 values increase platelet count decreases and vice versa. Thus IL-6 levels can be used in predicting severity of dengue fever and help in providing early treatment thus preventing mortality.

KEYWORDS- Interleukin-6, Dengue Fever, Platelet count, Severe Dengue Fever

CONTENTS

Sr. No.	Topic	Page No.
1.	INTRODUCTION	1-4
2.	OBJECTIVES	5
3.	REVIEW OF LITERATURE	6-21
4.	MATERIAL AND METHODS	22-30
5.	RESULTS	31-51
6.	DISCUSSION	52-56
7.	CONCLUSION	57
8.	LIMITATIONS AND SCOPE OF THE STUDY	58
9.	SUMMARY	59-60
10.	BIBLIOGRAPHY	61-67
11.	ANNEXURES	
	ANNEXURE I – CONSENT FORM	68-71
	ANNEXURE II – PROFORMA	72-75
	ANNEXURE III – MASTER CHART	76-78

LIST OF TABLES

TABLE. NO.	DESCRIPTION	PAGE NO.
1	Distribution of patients based on gender	31
2	Age distribution of dengue patients based on age group	32
3	Distribution of Day of illness on Day 1 and 4 of admission and severity of dengue at day 1 and day 4 of admission	33
4	Correlation between Day of illness at day of admission with severity of Dengue fever	35
5	Comparison of IL-6 Levels with Severity of dengue fever	37
6	Correlation between IL-6 levels at Day1 of admission and Platelet count at different days of admission	38
7	Correlation between IL-6 levels at Day4 of admission and Platelet count at different days of admission	41
8	Comparison of IL-6 Levels with Outcome of dengue fever	44
9	Comparison of IL-6 Levels with Hospital Stay	45
10	Comparison of IL-6 Levels with Inotropic Support	46
11	Comparison of IL-6 Levels with RDP Transfusion	47
12	Comparison of IL-6 Levels with SDP Transfusion	47
13	Diagnostic test results	48

LIST OF FIGURES

FIGURE NO.	DESCRIPTION	PAGE NO.
1	Clinical Course of Dengue	8
2	Risk factors of DHF	10
3	The 2009 revised dengue classification	11
4	Pathogenesis of dengue fever	12
5	Proposed model for the pathogenesis of DF, DHF, and DSS, based on an integrated view of the data	16
6	Dengue diagnosis	18
7	Standard Preparation	26
8	Reagents of IL-6 ELISA Kit	28
9	ELISA Microplate Reader	28
10	Distribution of patients based on the gender	31
11	Age distribution of dengue patients based on age group	32
12	Distribution of Severity of dengue on Day1 and 4 of admission	34
13	Correlation between Day of illness at Day 1 of admission with severity of Dengue fever	36
14	Correlation between Day of illness at Day 4 of admission with severity of Dengue fever	36
15	Correlation between IL-6 levels on Day1 of admission with Platelet count on Day 1 and Day 2 of admission	39
16	Correlation between IL-6 levels on Day1 of admission with Platelet count on Day 3 and Day 4 of admission	39
17	Correlation between IL-6 levels on Day1 of admission with Platelet count on Day5 and Day6 of admission	40

18	Correlation between IL-6 levels on Day1 of admission with Platelet count on Day7 admission	40
19	Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day1 and Day2 of admission	42
20	Correlation between IL-6 levels on Day4 of admission with Platelet count on Day3 and Day4 of admission	42
21	Correlation between IL-6 levels on Day4 of admission with Platelet count on Day5 and Day6 of admission	43
22	Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day7 of admission	43
23	Relationship between Hospital Stay and IL-6 levels on Day 1 and Day 4 of admission	45
24	ROC curve for the diagnostic test of IL-6 levels Day1 of admission and Severity of dengue fever at Day1 of admission	50
25	ROC curve for the diagnostic test of IL-6 levels Day4 of admission and Severity of dengue fever at Day4 of admission	50
26	ROC curve for the diagnostic test of IL-6 levels Day1 of admission and Outcome (Expired)	51
27	ROC curve for the diagnostic test of IL-6 levels Day4 of admission and Outcome (Expired)	51

INTRODUCTION

Dengue infection is globally spread arboviral infection which is spread by the bite of Aedes genus mosquitoes (primarily Aedes aegypti, and sometimes Aedes albopictus), which are found in subtropical and tropical regions. The infection itself causes a variety of clinical manifestations, both non-severe and severe.¹ Incubation period of the infection ranges from 3-14 days (mean being 7 days).

The 4 antigenically different dengue virus serotypes that cause dengue fever are DENV-1, DENV-2, DENV-3, and DENV-4. All 4 have the potential to cause life threatening illness. Dengue virus being RNA virus belongs to Flaviviridae family/Flavivirus genus, which also includes the Japanese encephalitis virus, West Nile virus, Yellow Fever virus, and St Louis encephalitis virus.²

The virion is made up of 3 structural proteins along with the lipoprotein envelope and 7 non-structural proteins, the most important out of these seven non-structural protein is NS1(non-structural protein 1). Prior infection with any one of the serotypes results in lifelong immunity for that particular serotype; however, cross protection to other serotypes lasts only a few months.¹⁻³

Dengue infection threatens approximately two-fifths of the world's population (mainly those living in subtropical and tropical countries), or up to 2.5 billion people.² Approximately 50 million infections occur every year across the globe, with 0.5 million of them requiring hospitalization for dengue hemorrhagic fever. Children under the age of 5 years account for 90% of the cases.² However, epidemiology is changing both regionally and globally. Adults are more likely than children to contract classic dengue fever. Dengue Fever is now endemic in over 100 countries, primarily in South East Asia, the Western Pacific, and also the Americas.^{2,4-6} Severe manifestations, such as dengue shock syndrome and dengue hemorrhagic fever, and

other unusual manifestations, are becoming more common in previously unaffected areas.

Dengue fever epidemics have become more common in many parts of India since the 1990s. Between 1998 and 2009, 82,327 dengue cases were reported (incidence: 6.34 per million population). Dengue fever was observed in 2,13,607 cases (incidence: 34.81 per million population) during a more recent period (2010-2014).⁷

CLASSIFICATION

Dengue is classified into three levels of severity, according to the 2009 WHO criteria: dengue with no warning signs, dengue with warning signs (that are continuous vomiting, liver enlargement, abdominal pain, fluid accumulation, lethargy, mucosal bleeding, thrombocytopenia with rising hematocrit), and last being severe dengue (that includes severe bleeding, severe plasma leakage, or organ failure). The patients who become alright after defervescence generally have non-severe dengue, whereas the ones which deteriorate show warning signs. Generally, these people most likely recover after intravenous rehydration. But further deterioration, however, is termed as severe dengue, although recovery can be possible once it is timely diagnosed and adequately treated.¹

DENGUE FEVER

It is self-limiting that usually lasts 5-7 days. During the acute illness stage, it can be debilitating. Clinical features differ depending on the patient's age. Young children and infants may experience unexplained febrile illness with a maculopapular rash. In older children there can be a mild febrile disease or severe disease with nausea, vomiting, high grade fever (usually biphasic), retroorbital pain, severe headache, arthralgia, myalgia, and petechiae can occur in older children and adults.

Leukopenia and thrombocytopenia are common in people of all ages. DF may be associated with bleeding complications like epistaxis, gingival bleeding, hematuria, gastrointestinal bleeding, and menorrhagia (in women).⁸

DHF

It is distinguished by DF symptoms as well as thrombocytopenia, plasma leakage and hemorrhagic manifestations. A positive Hess test/Tourniquet test may indicate DHF; but it is currently being debated because of its low sensitivity/specificity. In DHF, disease severity is determined by plasma leakage. It is also the most significant distinction between DF and DHF. DHF is classified into 4 grades based on clinical manifestations and disease severity, with most severe being Grade 4. Many patients have fine petechiae on face, soft palate, axillae, and extremities, which is common during the febrile phase. At the end of a febrile phase the critical phase begins, and it is characterized by a rapid drop in temperature and is frequently accompanied by thrombocytopenia, plasma leakage and hemoconcentration.⁸

DSS

DSS occurs in extreme instances with major plasma loss and can be fatal if timely not intervened. It is characterized as weak pulse with narrow pulse pressure of 20 mm of Hg, cool clammy skin, and agitation. Patient might collapse within 12-24 hours of falling into shock, or he or she could recover quickly with volume replacement treatment.

PATHOGENESIS

A prevalent theory on the pathophysiology of severe dengue is the formation of a "cytokine storm," or changes in cytokine levels that might cause endothelial cell dysfunction.⁹ Endothelial cell vascular permeability will result in plasma leakage, which is indicative of DHF and DSS.

Several investigations have shown that the two primary pathophysiological alterations in Dengue infection are plasma leakage owing to increased vascular permeability and bleeding propensity due to hemostasis dysregulation.^{10,11}

Endothelial cell overproduction of cytokines and adhesion molecules during immune response results in coagulopathy in dengue infection. IL-6 is one of the inflammatory mediators involved in this disease.

Huang et al, discovered a substantial increase in production of IL-6 and IL-8 in patients with DSS/DHF in a research using dengue virus-infected HUVEC. IL-6 may enhance vascular permeability, fluid-phase endocytosis, and CNS-derived endothelial cell transcytosis. Furthermore, IL-6 may increase leukocyte recruitment and so play a role in local inflammatory responses. There is evidence that DV-infected endothelium cells secrete IL-6 and IL-8 locally, which may promote leukocyte recruitment and activate or harm endothelial cells.^{11,12}

OBJECTIVES

PRIMARY

To study IL-6 levels to predict the severity of dengue fever.

SECONDARY

To correlate IL-6 levels with platelet count in patients with dengue fever.

REVIEW OF LITERATURE

Dengue fever is a highly endemic infectious illness that is fast becoming a worldwide burden. Dengue fever ranges from moderate fever to shock syndrome and dengue hemorrhagic fever. Increasing air travel, globalization and unplanned urbanization have raised the risk of infection and helped dengue spread geographically and demographically.¹³

PRIMARY DENGUE INFECTION

Primary dengue infection occurs when an individual is exposed for the first time to any of the four dengue virus serotypes. It may or may not cause symptomatic infection. Increasing titers of IgM and IgG antibodies emerge 3 to 5 and 6 to 10 days after the commencement of infection, respectively, in initial infection. IgM is temporary, as it fades 2-3 months post onset of the disease, but IgG remains for life.¹⁴ As a result, there is life long immunity after initial infection against that certain serotype. However, cross protection is not provided against other serotypes.

SECONDARY DENGUE INFECTION

Classical DF is frequently caused by a subsequent infection by a previously unknown DENV serotype. 2-3% of secondary dengue progress to DHF, thus leading to DSS and mortality. During subsequent infection with other serotype, presence of modest levels of heterotypic antibodies enhances virus entry via Fc receptors to monocytes, resulting in increase of viral load and illness severity. This is referred to as Antibody Dependent Enhancement. Antibodies which are cross-reactive are produced against prM and fusion loop have been found to be weakly neutralizing, thus leading to increase in the infection.^{14,15} Even though antibody dependent

enhancement is related to illness severity, not all severe cases are linked with secondary infection, and not all secondary infections develop DHF/DSS. Cross-reactive memory T cells, in addition to humoral immunity, may play a role in either providing protective immunity or generating immunopathology.¹⁶

CLINICAL COURSE

Dengue fever is distinguished by abrupt onset of fever, headache (usually behind the eyes), muscle and joint discomfort, and a rash.¹ Dengue fever is often known as breakbone fever because of the accompanying muscle and joint symptoms.^{17,18} The infection progresses through three stages: febrile phase, critical phase, and recovery phase.¹ The febrile stage is characterised by high temperature, potentially exceeding 40°C (104°F), as well as widespread discomfort with headache; which typically lasts 2-7 days.^{17,18} Vomiting is also possible. Rash appears in 50% to 80% of people experiencing symptoms. First 2 days there is flushing of the skin, or later during 4th -7th day of the illness maculopapular rash appears. A rash has also been characterised as "islands of white amid a sea of red." Some petechiae, as well as modest bleeding from the mouth, nose and mucous membranes, may emerge at this time. The fever is biphasic or "saddleback," breaking and then returning for one or two days.¹⁷⁻²¹

As the fever subsides, some people's sickness progresses to a critical stage.²⁰ This phase is distinguished by considerable, diffuse plasma leakage that normally lasts 1-2 days. It can cause hypovolemia, ascites, pulmonary edema, and shock.¹ Organ malfunction and significant bleeding, usually from the gastrointestinal system, are also possible. DSS and DHF occur in <5% of all dengue cases; however, people who were previously infected with different serotypes of dengue virus (and so are

suffering a secondary infection) are at higher risk. Although rare, Critical phase is mostly seen in children and young adults.^{22,23}

Following the critical phase, the recovery phase begins, with reabsorption of the already leaked fluid back into the bloodstream over a time span of 2-3 days. The improvement is frequently noticeable, and can be accompanied by acute itching and bradycardia. Another rash may arise, either vasculitic/maculopapular in appearance, which is followed by desquamation.²²

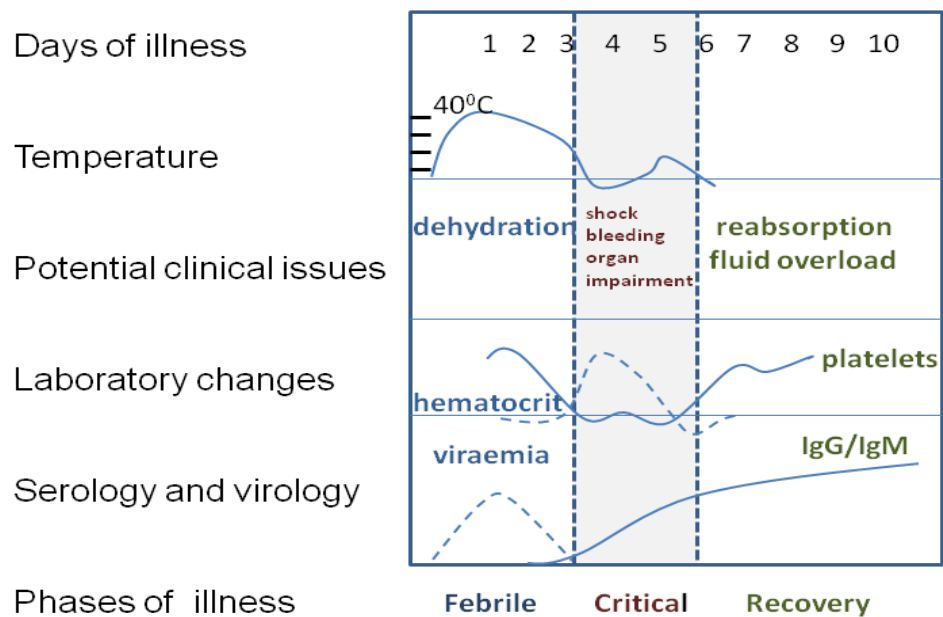


Figure 1: Clinical Course of Dengue

CLINICAL PRESENTATION OF DENGUE

According to expert groups, DF has multiple clinical presentations, and dengue patients have variety of clinical manifestations that vary depending on age and severity. Infection with either of the 4 dengue serotypes can cause asymptomatic dengue fever (DF) or more severe forms of the disease, such as hemorrhagic fever (DHF) and dengue shock syndrome (DSS).¹⁸ During the acute phase, presence of NS1 antigen which is a non-structural protein in the blood can be used to confirm dengue infection.²⁴ The sickness occurs quickly after an incubation period, progressing through 3 stages: i.e. febrile phase, critical phase, and recovery phase.¹ Adults and teenagers are more likely to be affected by DF, which can manifest as either a moderate fever or a more incapacitating illness.

DF is distinguished by the development of a high temperature, retro-orbital discomfort, intense headache, myalgia, rash and arthralgia, all at once. These symptoms are most common in the febrile phase.²² During the crucial period, the skin flushes and a petechial rash appears. This commonly happens during defervescence, ranging from 3rd to 7th day, that is accompanied with bleeding and capillary leakage.⁴ DSS/DHF mostly affects children under the age of 15, however it can affect adults as well.²¹ DHF is distinguished by a transitory increase in vascular permeability, which results in plasma leakage, as well as high fever, haemoconcentration, thrombocytopenia, and bleeding, which leads to DSS.^{13,25}

However, distinguishing DHF from DF and other viral infections, such as typhoid fever, can be challenging, especially during the febrile phase of the disease.²² Although secondary infection is thought to be the most important risk factor for DHF,

the interplay of virus, epidemiological risk factors and the host influences the incidence of DHF epidemics.^{13,25}

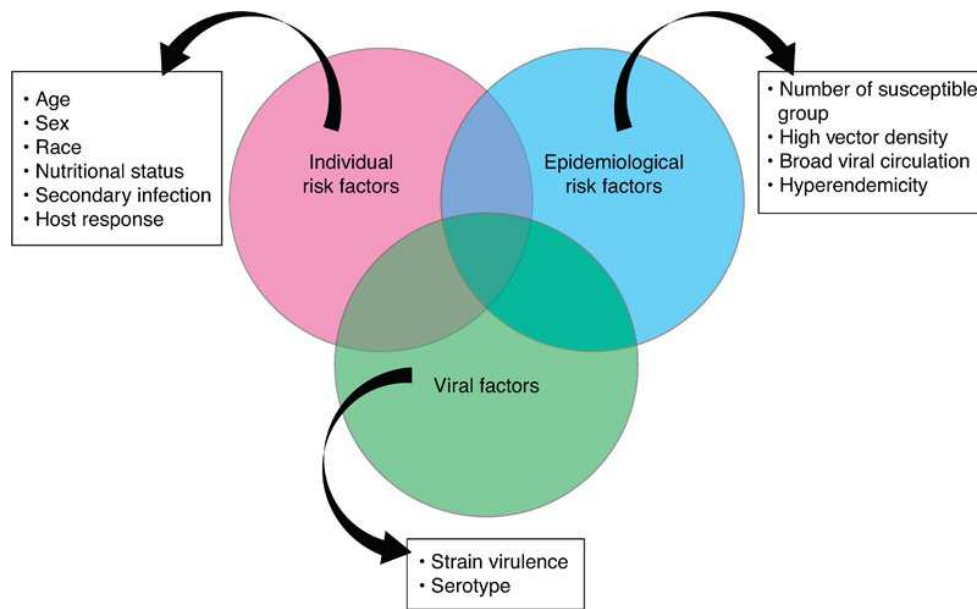


Figure 2: Risk factors of DHF

DENGUE CLASSIFICATION

Dengue is classified into three levels of severity, according to the 2009 WHO criteria: dengue with no warning signs, dengue with warning signs (that are continuous vomiting, liver enlargement, abdominal pain, fluid accumulation, lethargy, mucosal bleeding, thrombocytopenia with rising haematocrit), and last being severe dengue (that includes severe bleeding, severe plasma leakage, or organ failure). The patients who become alright after defervescence generally have non-severe dengue, whereas the ones which deteriorate show warning signs. Generally these people most likely recover after intravenous rehydration. But further deterioration, however, is

termed as severe dengue, although recovery can be possible once it is timely diagnosed and adequately treated.¹

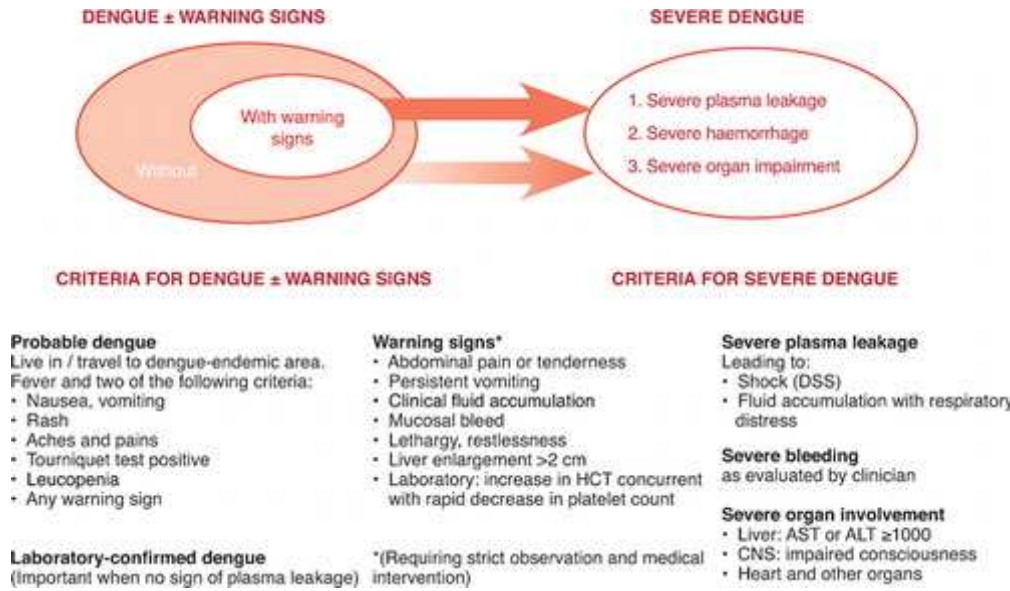


Figure 3: The 2009 revised dengue classification¹

ETIOPATHOGENESIS

Dengue Fever being a severe entity affects individuals of all age groups.²⁵ It is transmitted mainly by *Aedes aegypti* and spreads mainly in the rainy season.²⁶

Etiologies of dengue fever are:

- Direct skin infection by the virus²⁷
- Viral replication, primarily in macrophages²⁸
- Virus-host interaction inducing chemical and immunologic response²⁷

Dengue virus enters the host organism through the skin after being bitten by an infected mosquito. The course of the sickness is aided by innate, cellular, and humoral host immune interactions, and the more severe clinical indications appear when the virus is quickly cleared from the body of the host. Thus, even large viral loads are not responsible for severe clinical manifestation during the infection.²⁹ Changes in endothelial microvascular permeability and thromboregulatory systems cause an increase in protein and plasma loss. Some theories propose that, plasma leakage is mediated by endothelial cell activation produced by the complement system, monocytes, T-cells, and different inflammatory chemicals. Thrombocytopenia might be caused by changes in megakaryocytopoiesis, as evidenced by infection of human hematopoietic cells and impaired progenitor cell development. This can result in platelet malfunction, injury, or depletion, which can lead to major haemorrhages.^{2,6}

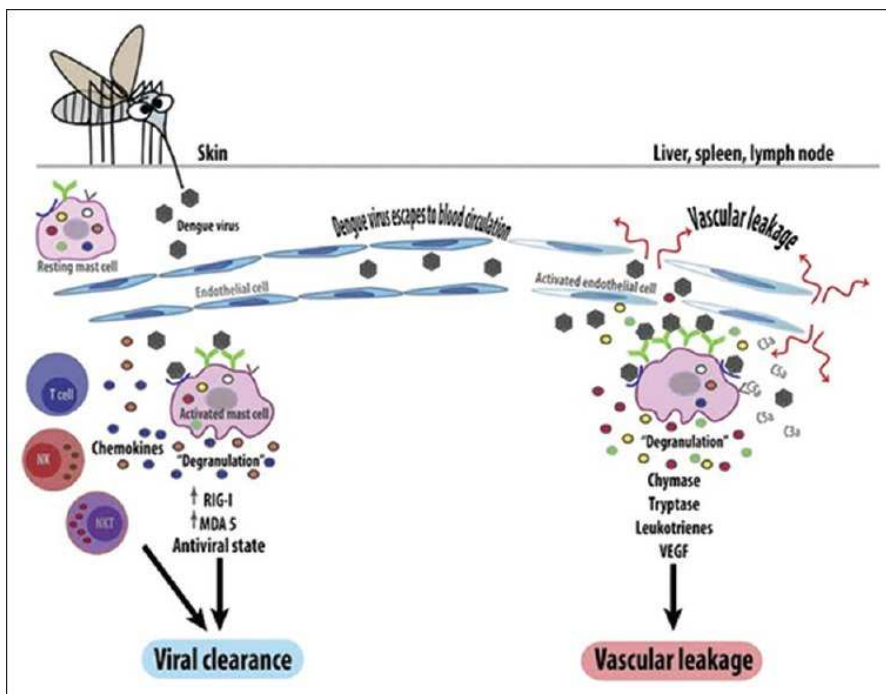


Figure 4: Pathogenesis of dengue fever

IL-6: FUNCTION RELATIONSHIPS

Interleukin-6 (IL-6) is an exceedingly diverse immunomodulatory cytokine generated after infection, trauma, and immunological challenge by mast cells, fibroblasts, endothelial cells, monocytes, and both benign and malignant lymphocytes of B and T cell origin.³⁰ It is important in host defence because of its diverse immunological and hematopoietic actions, as well as its capacity to trigger the acute phase response.^{2,6,30-33}

The stimulation of these acute phase reactions by IL-6 has been viewed as part of an effort to preserve homeostasis, as well as being engaged in the control of other aspects of inflammation, primarily cytokine responses and tissue inflammatory infiltration. In this sense, serum IL-6 levels appear to correlate with mortality in DSS patients.^{30,34}

In DV infection, an increased serum IL-6 level may also be related with the production of antiplatelet, antiendothelial autoantibodies, coagulation abnormalities leading to haemorrhage, and plasma leakage.³⁵

Indeed, IL-6 enhances inflammatory responses by expanding and activating T cells, differentiating B cells, and inducing acute-phase reactants (C-reactive protein) by hepatocytes.

Increased IL-6 and IL-8 levels in DSS/DHF patients' sera and supernatants of DV-infected HUVEC demonstrated the role of these cytokines in the progression of bleeding and shock syndrome.¹¹ Suharti et al, also found increase IL-6 in Dengue shock syndrome (DSS) non survivors.³⁴

PLASMA LEAKAGE IN DENGUE INFECTION

The predominant pathophysiologic anomaly observed in DSS/DHF is an abruptly increasing vascular permeability, which results in plasma leakage into the extravascular compartment. The pathogenesis mechanism underlying the increase in vascular permeability that is observed in DSS and DHF is unknown, but chemical mediators and cytokines such as tumour necrosis factor (TNF), Interleukin-1, Interleukin-2, Interleukin-6, complement activation products C3a and C5a, platelet-activating factor (PAF), and histamine have been implicated.

Kurane and Ennis proposed that fast increases in levels of mediators (such as TNF, IL-6, IL-2, IFN- γ , C3a, C5a, PAF, and histamine) produce increased plasma leakage, vascular permeability, and shock.³⁶

Higher the dengue virus infection by monocytes/macrophages, leads to increased activation of T-cells, which in turn results in increased quantities of chemical mediators and cytokines release.

COAGULOPATHY IN DENGUE VIRUS INFECTION

If the dysregulation of coagulation and fibrinolysis continues, hemostasis is maintained. The increased concentration of IL-6 may play an important role in the augmented generation of auto anti-platelet antibodies, raised levels of tissue plasminogen activator, and coagulation factor XII insufficiency in the intrinsic route.¹⁸ In acute stage of DSS/DHF, hyperfibrinolysis is produced by elevated tPA generation. Statistical analysis reveals a significant relationship between high IL-6 levels and tissue plasminogen activator in DHF but not in DF. Infection with the Dengue virus causes endothelial synthesis of tPA and IL-6. Anti-IL-6 antibodies inhibit tPA de novo synthesis, demonstrating that tPA generation by endothelial cells is IL-6 dependent.

IL-6 can inhibit the production of coagulation factor XII, the first factor to activate the intrinsic coagulation cascade. APTT prolongation in DHF patients due to a lack of intrinsic route is most likely related to reduced coagulation factor production.

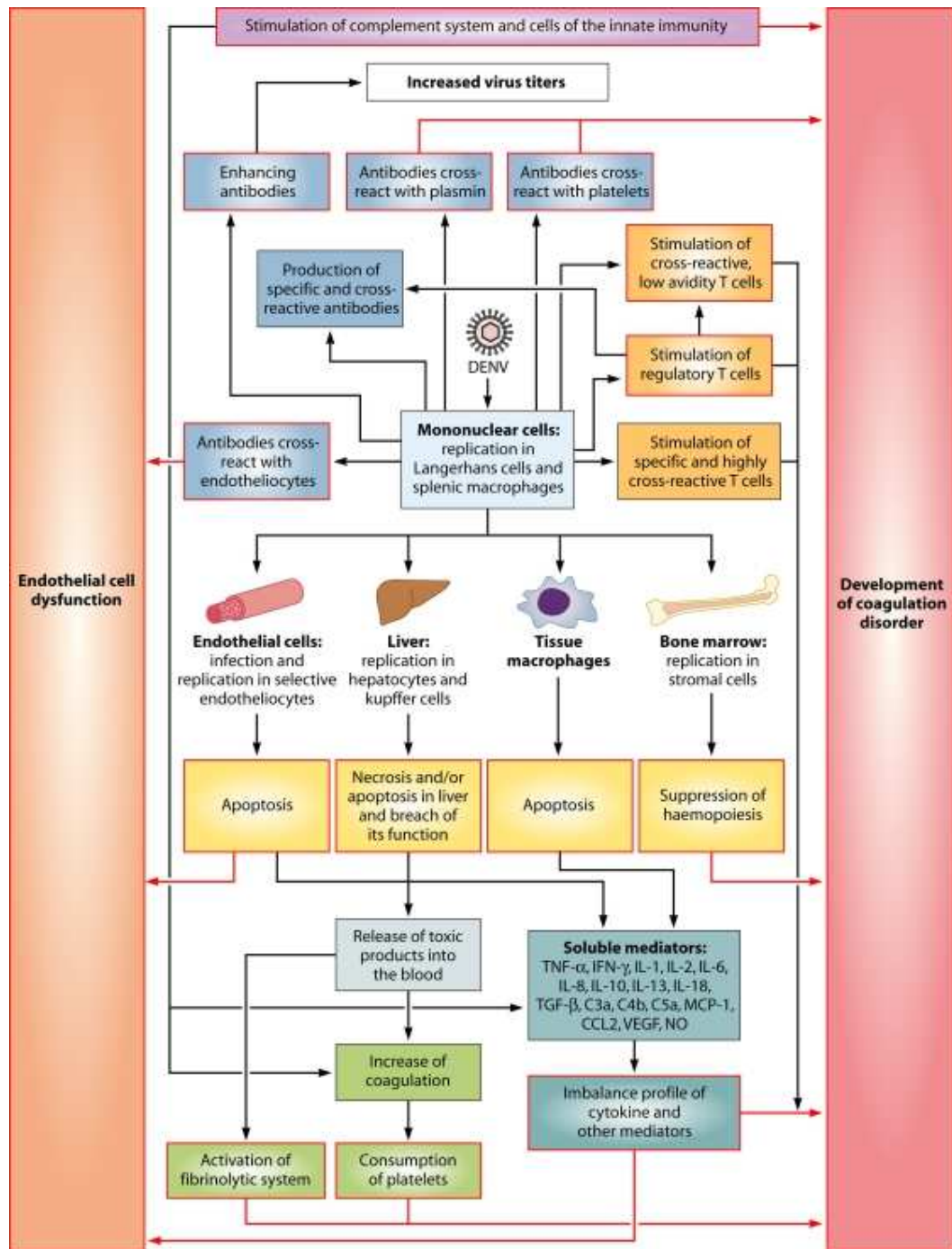


Figure 5: Proposed model for the pathogenesis of DF, DHF, and DSS, based on an integrated view of the data

CLINICAL MANIFESTATIONS

Undifferentiated fever

This stage is most common during the original infection, although it can also occur after secondary infection. This frequently goes undetected because of other viral illnesses mimicking it.

Dengue fever

It is most common in older children and adults and occurs after both primary and secondary dengue infection. Symptoms include high grade biphasic fever that lasts 3 days to 1 week.^{37,38} Other reported symptoms include severe headache (mostly retrobulbar), lassitude, myalgia and aching joints, metallic taste, appetite loss, abdominal pain, diarrhoea, vomiting,. Dengue fever is often known as breakbone fever due to the accompanying myalgia and joint discomfort.^{17,29} 50-82% of DF patients present with cutaneous rash. Initially the rash is due to capillary dilatation which results in transient flushing of the face within first two days of fever. Then within 3 days to 1-week secondary rash appears which is morbilliform or maculopapular but asymptomatic. Individual rashes may coalesce and then look like large confluent erythematous patches with pinpoint bleeding spots and spherical islands of sparing, creating the image of "white islands in a sea of crimson."^{29,39} Cutaneous rash along with pruritis is often asymptomatic, and occurs in just 16-27% patients. Although gingival bleeding and epistaxis, petechiae/purpura, significant menstruation, and gastrointestinal tract (GIT) haemorrhage are uncommon in DF, they can occur.^{40,41}

Dengue shock syndrome

Dengue Haemorrhagic Fever with a weak pulse and narrow pulse pressure (20 mmHg), restlessness, chilly, circumoral cyanosis and cold clammy skin, is characterised as DSS. The significant death rate associated with DSS is due to progressive worsening shock, DIC and multiorgan dysfunction. After the shock part is corrected patient recovers with supportive care.^{42,43}

DIAGNOSTIC APPROACHES

Viremia detection- Viraemia can be detected 24-48 hours prior to the starting of the fever and is present for 5-6 days. In this meanwhile, NS1 antigen, virus's RNA, the virus itself, may be found in patient's blood, plasma, and serum, as well as from tissues in some of the fatal cases.⁵¹ For epidemiological purposes and clinical diagnosis purposes, virological, molecular, and serological approaches are utilised.

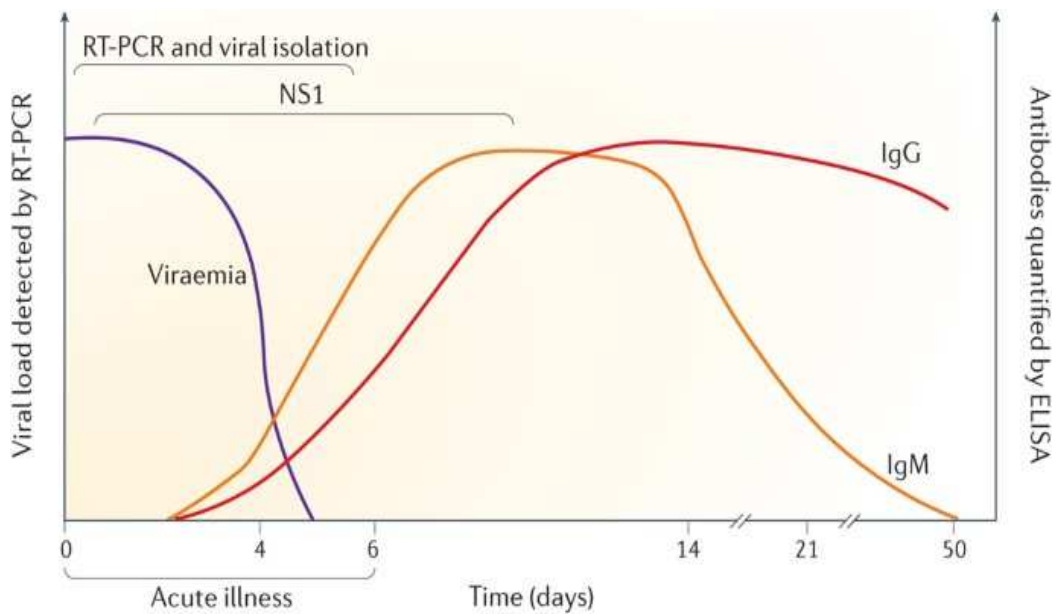


Figure 6: Dengue diagnosis⁴⁴

The most often used test in ordinary practice is the detection of anti-DENV IgM antibodies. To confirm infection, anti-Dengue virus IgM titres are quantified in the acute phase of sickness in patient's sera, whereas individuals in convalescence are identified by IgM and IgG seroconversion by comparing antibody titres in matched acute and convalescent sera. Anti-Dengue virus IgM antibodies detected in samples obtained on day 6 of acute symptoms can provide a provisional diagnosis for individuals suspected of having dengue. There are commercial kits available for IgM or IgG detection in enzyme-linked immunosorbent assay (ELISA) and less sensitive fast test formats.

Reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, DENV isolation in mosquito cell lines, and mosquito inoculation all help to confirm and identify the agent virologically. Though isolation of the virus and identification is extremely specific, but it has limited sensitivity and is both time as well as resource consumption. Detection of DENV RNA, on the other hand, provides a quick, specific and sensitive approach for diagnostic confirmation of the virus. The identification of NS1 protein opens the door to early diagnosis of the infection. NS1 detection sensitivity and specificity are affected by the time of sample collection, the infecting serotype, primary vs secondary dengue infection, and the test format.⁴⁴

RELEVANT STUDIES

Suharti C, Hack CE, et al. found that IL-6 is substantially linked with activation indicators of both coagulation and fibrinolysis in a prospective clinical trial of 50 patients with Dengue Shock Syndrome. In this study, IL-6 levels in Dengue Shock Syndrome were elevated (non survivors).³⁴

Huang et al discovered a considerable rise in Interleukin-6 and Interleukin-8 production in patients with DSS/DHF in a research of dengue virus infected HUVEC (Human Umbilical Vein Endothelial Cell). Increased Interleukin-6 and Interleukin-8 levels in DSS/DHF patients' sera and supernatants in a prior study demonstrated the role of these cytokines in the progression of haemorrhage and shock syndrome.⁴⁵

Fariha Kanwal et al found that serologically Dengue Virus 2 positive serum samples from DHF and DF groups had differently enhanced IL-6 expression. The control group had a mean level of IL-6 expression of 6.72pg/ml in healthy patients, but the DHF and DF groups had mean levels of Interleukin-6 expression of 150 and 591.59pg/ml, respectively. The DF group had considerably lower levels of IL-6 expression than the DHF group.

Abraham Varghese et colleagues found that TNF Alpha and IL-6 are linked to dengue illness severity, implying that they might be utilised to predict the disease severity. The level of SGOT was associated to IL-6 (Serum glutamic oxaloacetic transaminase).⁴⁶

In a study of Lei HY et al, cytokines such as Interleukin-6, Interleukin-8 and RANTES, are elevated after dengue virus infection. Interleukin-6 and Interleukin-8 serum levels were higher in DSS/DHF than in DF patients.

In a study Lien-Cheng Chen et al, all DHF non-survivors showed significantly larger increases in serum MIF (Macrophage migration inhibitory factor), IL-6, and IL-10, but not IFN- γ or TNF- Alpha, than did Dengue fever patients. ¹⁷

In a study Imad HA et al, significantly elevated levels of IL-6 were observed during the acute phase in patients with bleeding, with a background of plasma leakage. ⁴⁷

MATERIAL AND METHODS

Study Centre: Department of Pediatrics, J N Medical College and Hospital, Belgaum's KAHER Dr. Prabhakar Kore Hospital PICU, where pediatric patients hospitalized with dengue were studied.

Study duration and period: One year (January 2021 - December 2021).

Study type: Cross sectional Observational Study was used as the study design.

Subjects include: The study population including pediatrics age group 6months-18years is selected on the basis of the NS1 or IgG or IgM positive report.

Sample size:

Formula used for sample size calculation:

$$n = [(Z_{\alpha/2} + Z_{\beta}) / C]^2$$

Where C is, $C = 0.5 \times \log_e [(1 + r) / (1 - r)]$

In the above formula, r is the correlation coefficient, at 95% confidence level $Z_{\alpha/2}$ is 1.96 and Z_{β} value for 90% power is 1.2815.

With 80% power, 95% confidence level and with above correlation coefficient, sample size required is $56.992 \approx 57$. Minimum sample size required is 57 subjects. Larger the sample size, better the precision.

So, Sample Size=60

Data Collection:

The research involves pediatrics age group 6 months-18years is selected on the basis of the NS1 or IgG or IgM positive report.

The serum sample is collected on 1st and 4th day of admission. Sera are separated from whole blood by centrifuging it at 3600r/minute for 8 minutes and then until the detection of IL-6 levels employing ELISA kit, it is stored at -80 degree Celsius.

The patients are classified on the basis of WHO criteria into severe and non-severe dengue.

Inclusion Criteria:

All Dengue NS1 Antigen or IgG or IgM positive patients.

Exclusion Criteria:

1. Patients without confirmed diagnosis of dengue infection.
2. Patients who are known cases of Rheumatoid Arthritis, Systemic Lupus Erythematosus, Ankylosing Spondylitis, and Inflammatory bowel disease.

Data Collection and Monitoring:

The study population including pediatrics age group 6months-18years is selected on the basis of the NS1 or IgG or IgM positive report.

The serum sample is collected on 1st and 4th day of admission. Sera is separated from whole blood by centrifuging it at 3600 r/minute for 8 minutes and stored at -80 degree Celsius. The samples are then subjected to 1:3 dilution until the

detection of IL-6 levels employing ELISA kit by Krishgen Biosystems. All the samples are then run in doublets and IL-6 levels are used for evaluation.

Then the patients are evaluated for 7 days on the basis of –

1. Platelet Count
2. Packed Cell Volume
3. Blood Pressure (Systolic Blood pressure and Diastolic Blood Pressure)
4. Temperature
5. Pulse rate
6. Urine output
7. Organ Involvement

ELISA TESTING FOR HUMAN IL-6 GENLISA (KRISHGEN BIOSYSTEM)

Reagent Preparation

1. **(20X) Wash Buffer:**

Dilution: To make Wash Buffer (1X), add 5ml of Wash Buffer (20X) to 95ml of DI water. This is the working solution

2. **(1X) Assay Diluent:** Ready to use solution.

3. **Standard Lyophilized (Recombinant Human IL-6, 1 μ g/ml) (lyophilized):**

Reconstitute lyophilized human IL-6 standard with 20 μ l of distilled water to achieve final concentration 1 μ g/ml. Mix gently and leave to stand for 15 minutes before further dilution. Use immediately for the experiment.

Dilution 1: Thaw and Dilute the recombinant protein by adding 5 μ l of standard solution in 495 μ l of Assay Diluent (1X) to prepare 0.5 ml of 10 ng/ml.

Dilution 2: Add 20 μ l of 10ng/ml to 480 μ l Assay Diluent (1X) to prepare top standard of 400pg/ml

4. **Biotin Conjugated Detection Antibody (50 μ l):**

Dilution: Add 20.83 μ l of Detection Antibody to 4979.17 μ l of Assay Diluent (1X) to make final volume to 5ml.

5. **Concentrated Streptavidin-HRP (15 μ l):**

Dilution: Add 5 μ l of Streptavidin-HRP to 4995 μ l of Assay Diluent (1X) to make final volume to 5ml.

Assay procedure

1. Prior to use all reagents to be brought to room temperature. All samples and standards are strongly recommended to be run in duplicates. For each assay a standard curve is required.
2. Standards Preparation: The lyophilized vial has to be reconstituted with 20 μ l of Distilled water to generate a 1 μ g/ml. Keep the standard for 15 mins with gentle agitation before making further dilutions. Dilute 5 μ l of original Standard (1 μ g/ml) with 495 μ l of Assay diluent (1X) to generate a 10 ng/ml middle stock solution. By using main stock solution perform serial dilutions as per the below table. Thus, the Human IL-6 Standards concentration are 400 pg/ml, 200 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 12.5 pg/ml and 6.25 pg/ml. Assay Diluent (1X) serves as the zero standard (0 pg/ml).

Standard Concentration	Standard No	Dilution Particulars
1 μ g/ml (Lyophilized)	Standard Main stock	Original Standard. Reconstitute in 20 μ l of Distilled water
10 ng/ml	Middle stock	5 μ l Original Standard + 495 μ l Assay Diluent (1X)
400 pg/ml	Standard No.8	20 μ l Middle Stock + 480 μ l Assay Diluent (1X)
200 pg/ml	Standard No.7	250 μ l Standard No.8 + 250 μ l Assay Diluent (1X)
100 pg/ml	Standard No.6	250 μ l Standard No.7 + 250 μ l Assay Diluent (1X)
50 pg/ml	Standard No.5	250 μ l Standard No.6 + 250 μ l Assay Diluent (1X)
25 pg/ml	Standard No.4	250 μ l Standard No.5 + 250 μ l Assay Diluent (1X)
12.5 pg/ml	Standard No.3	250 μ l Standard No.4 + 250 μ l Assay Diluent (1X)
6.25 pg/ml	Standard No.2	250 μ l Standard No.3 + 250 μ l Assay Diluent (1X)
0 pg/ml	Standard No.1	500 μ l Assay Diluent (1X)

Figure 7: Standard Preparation

3. Add 100 µl of Standards and Samples to the plate. Seal plate and incubate for 2 hours at Room Temperature (18-25°C).
4. Aspirate and wash plate 5 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Then all the serum samples are diluted in 1:3 dilution by adding 80 µl of serum with 160 µl of Assay Diluent.
6. Add 100 µl of diluted Detection Antibody solution to each well, seal plate and incubate for 1 hour at Room Temperature (18-25°C).
7. Wash the ELISA plate 5 times with the Wash Buffer (1X) as in step 4.
8. Add 100 µl of diluted Streptavidin: HRP solution to each well, seal plate and incubate for 15 minutes at Room Temperature (18-25°C).
9. Wash plate 5 times with Wash Buffer (1X) as in step 4.
10. Add 100 µl of TMB Substrate solution and then incubate it in the dark for 30 minutes at Room Temperature. Positive wells will turn blue in colour. Sealing the plate in this step is not necessary.
11. Stop the reaction by adding 100 µl of the Stop Solution to each and every well.
The wells that are positive will become blue from yellow.
12. Read the absorbance at 450 nm within 30 minutes of stopping the reaction.
13. All the samples are run in doublets and the mean value of IL-6 is calculated and used for the study.



Figure 8: Reagents of IL-6 ELISA Kit



Figure 9: ELISA Microplate Reader

Sensitivity: The Limit Of Detection: It is the lowest detectable concentration which corresponds to a signal of Mean of '0' standard plus 2* SD. Ten replicates of '0' standards were evaluated and the LOD was found to 5 pg/ml.

Specificity: The antibodies used in the kit for detection and capture are monoclonal antibodies specific for human IL-6. No cross reaction was observed in presence of 50ng of IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, GM-CSF, IFN alpha, IFN gamma, LIF, MIP-1alpha, MIP-1beta, MCP-1, OSM, RANTES, TGF beta, TNF alpha, TNF beta. Very low cross reactivity was observed with G-CSF.

Dynamic Assay Range: 6.25 pg/ml to 400±20 pg/ml. The serum samples were diluted in 1:3 dilution thus making the maximum assay range to be ≥1260pg/ml.

Precision: Intra-Assay: CV<12%

Reference Values: Serum samples from 34 healthy individuals with low CRP levels were evaluated for IL-6 using this ELISA kit. The samples had results in range between 0 to 50 pg/ml. 31 samples obtained value below 17 pg/ml.

Proforma for Data Collection: Data was gathered using the proforma provided in the annexure.

STATISTICAL ANALYSIS:

Data is analyzed using statistical software R version 4.1.2. To compare the IL-6 levels with severity of dengue fever Mann-Whitney test was used. Spearman correlation (ρ) was used to check the correlation between IL-6 Levels and Platelet count. To check the normality of variables Shapiro-Wilk's test and box-plot is used. P-value less than or equal to 0.05 indicates statistical significance. To find the cut point of IL-6 levels at day1 and day4 with respect to severity of dengue and outcome(expired) diagnostic test is performed and Sensitivity, specificity, NPV and PPV were reported.

DATA VISUALIZATION:

MS Excel and MS Word were used to create a variety of graphs, including bar diagrams, pie diagrams, and line diagrams. After applying all statistical procedures, a p value (probability that the result is true) of 0.05 was judged statistically significant.

RESULTS

This present study aimed to assess the IL-6 levels in predicting the severity of dengue fever. Present study included total of 60 patients fulfilling inclusion criteria.

Table 1: Distribution of patients based on the gender

		Number	Percentage
Gender	Male	36	60%
	Female	24	40%
Total		60	100%

Among all the dengue patients, males were 60% and females were 40%.

Figure 10: Distribution of patients based on the gender

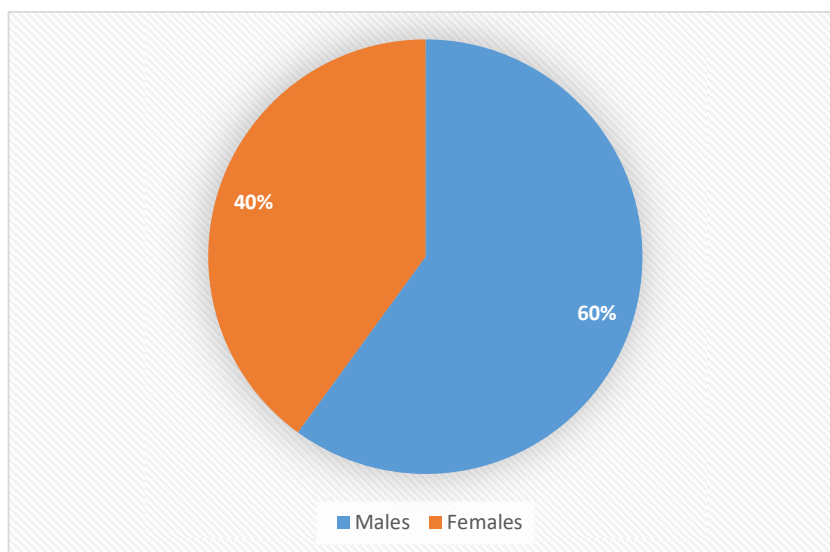


Table 2: Age distribution of dengue patients based on age group

Age (Years)	Number	Percentage
<1	3	5%
1-5	9	15%
6-10	16	26.7%
11-15	23	38.3%
16-18	9	15%
Total	60	100%

Among all the dengue patients 3 were in <1 years of age category, 9 were in 1-5 years age category, 16 were in 6-10 years age category, 23 were in 11-15 years age category and 9 were in 16-18 years age category.

Figure 11: Age distribution of dengue patients based on age group

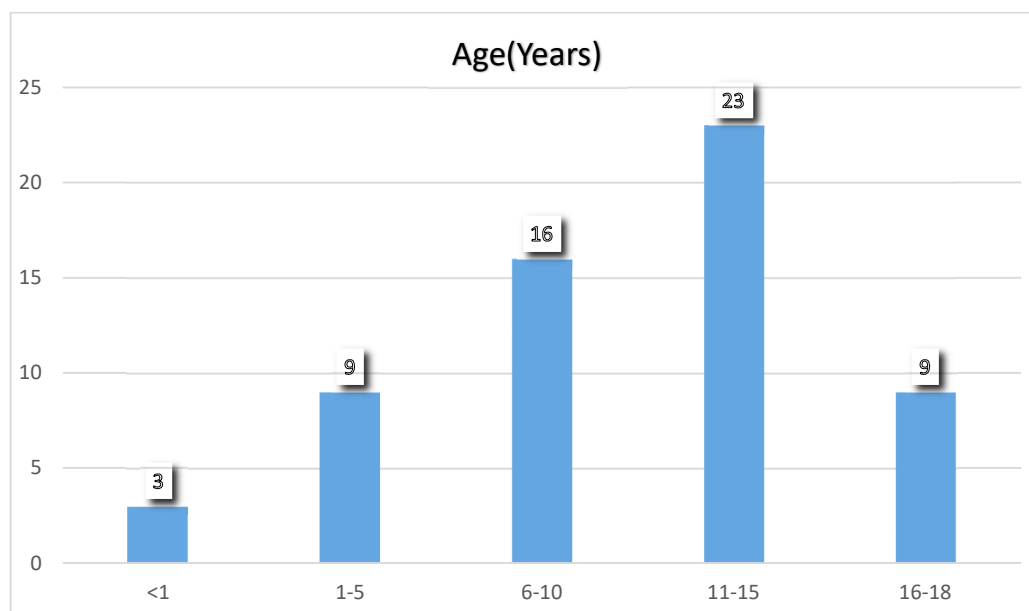


Table 3: Distribution of Day of illness on Day 1 and 4 of admission and severity of dengue at day 1 and day 4 of admission.

	Day of Illness	Number of subjects (%)
Day 1 of admission	1	2 (3.33%)
	2	13 (21.67%)
	3	9 (15%)
	4	18 (30%)
	5	13 (21.67%)
	6	5 (8.33%)
Day 4 of admission	4	2 (3.33%)
	5	13 (21.67%)
	6	9 (15%)
	7	14 (23.33%)
	8	11 (18.33%)
	9	5 (8.33%)
Severity at Day 1 of admission	Non-severe	45 (75%)
	Severe	15 (25%)
Severity at Day 4 of admission	Non-severe	44 (73.33%)
	Severe	10 (16.67%)
	Deaths	6 (10%)

Out of 60 dengue patients admitted, 30% had Day 4 of illness of Dengue Fever on the day of admission. On the Day of admission out of 60 patients, 45(75%) belonged to Non- Severe Dengue category and 15(25%) belonged to Severe Dengue. Similarly, on Day 4 of admission out of 60 patients, 44(73.33%) belonged to Non- Severe Dengue category and 10(16.67%) belonged to Severe Dengue and 6(10%) expired till Day 4 of admission.

Figure 12: Distribution of Severity of dengue on Day 1 and 4 of admission.

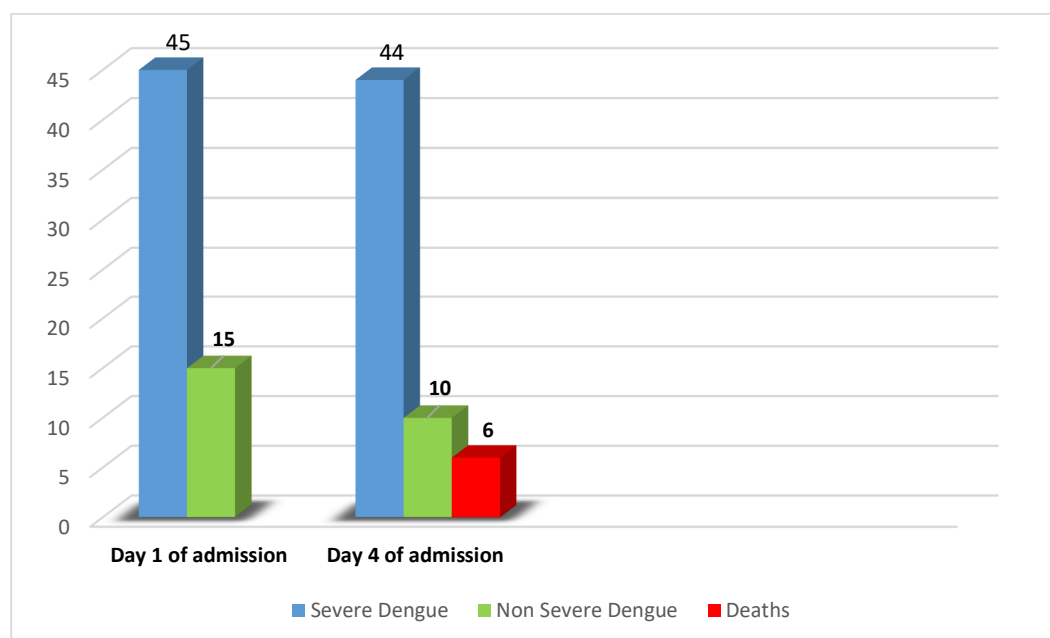


Table 4: Correlation between Day of illness at day of admission with severity of Dengue fever

	Day of Illness	Severity		p-value
		Non severe	Severe	
Day 1 of admission	1	2 (100%)	0 (0%)	0.003498*MC
	2	12 (92.31%)	1 (7.69%)	
	3	9 (100%)	0 (0%)	
	4	8 (44.44%)	10 (55.56%)	
	5	9 (69.23%)	4 (30.77%)	
	6	5 (100%)	0 (0%)	
Day 4 of admission	4	2 (100%)	0 (0%)	0.3443
	5	12 (92.31%)	1 (7.69%)	
	6	8 (88.89%)	1 (11.11%)	
	7	9 (64.29%)	5 (35.71%)	
	8	8 (72.73%)	3 (27.27%)	
	9	5 (100%)	0 (0%)	

Abbreviations: MC: Monte-Carlo’s simulation used in Chi square test

Out of 60 patients who got admitted with dengue fever, on the Day of admission, 18 patients had Day 4 of illness out of which 10 (55.56%) patients had Severe Dengue. By using Monte- Carlo’s simulation in chi-square test, there is significant association present between day of illness on the Day of admission with severity of dengue fever, p value being 0.003498. Also, 30% of the patients who presented to hospital on the Day of admission had Day 4 of illness and in that 55.56% had Severe Dengue.

Figure 13 : Correlation between Day of illness at Day 1 of admission with severity of Dengue fever

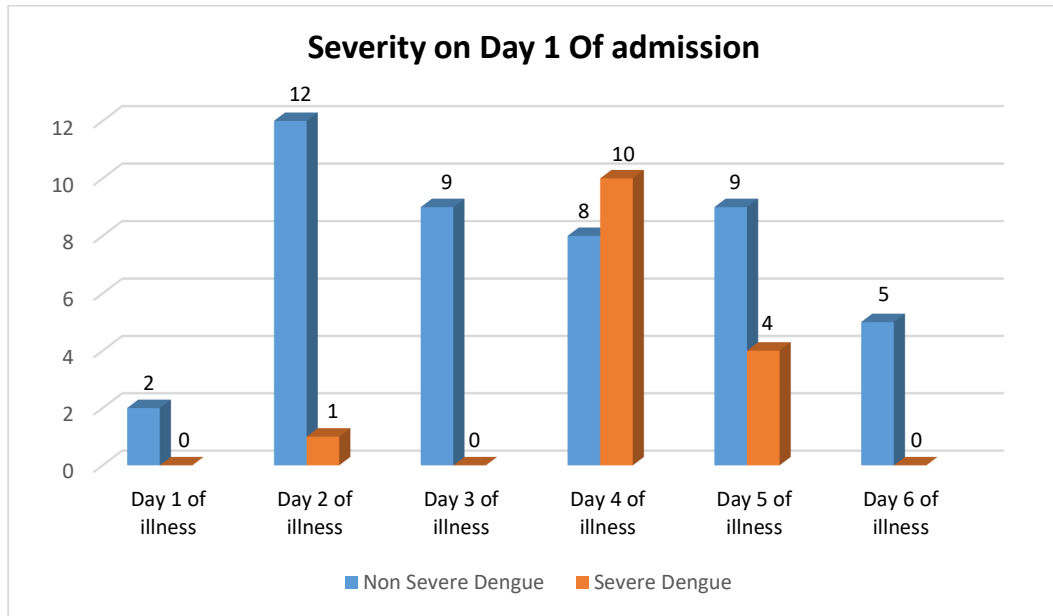


Figure 14: Correlation between Day of illness at Day 4 of admission with severity of Dengue fever

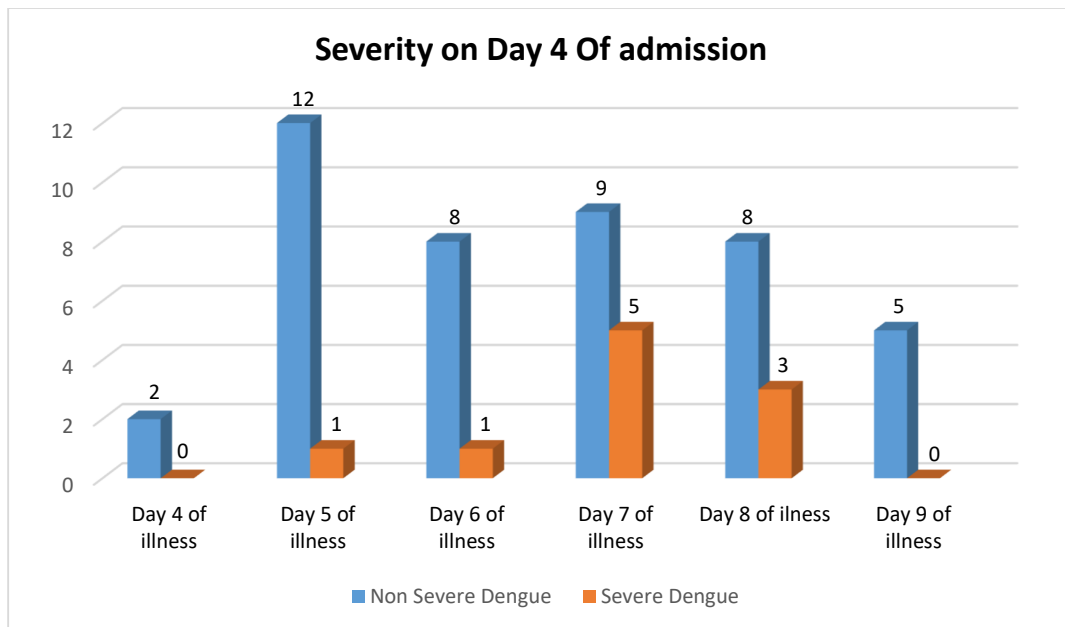


Table 5: Comparison of IL-6 Levels with Severity of dengue fever

		Severity of dengue fever	Severe	Non-Severe	p-value
IL-6 levels (pg/ml)	Day 1 of admission	Mean±SD	616.1680± 449.0418	312.1178 ± 397.4590	0.0176*
		Median (Min, Max)	675.2175(13.97, 1260)	150.2730(0, 1260)	
	Day 4 of admission	Mean±SD	747.2799 ± 558.9062	197.1161 ± 250.8504	0.0025*
		Median (Min, Max)	925.6268(0, 1260)	97.9095 (0, 1213.807)	

By Mann-Whitney test, there is significant difference in the distribution of IL-6 Levels at Day1 and Day 4 of admission between the groups. On the Day of admission Mean IL-6 value corresponding to Severe Dengue fever is 616.1680± 449.0418 pg/ml, and to Non-Severe Dengue fever is 312.1178 ± 397.4590 pg/ml. Similarly, on Day 4 of admission Mean IL-6 value corresponding to Severe Dengue fever is 747.2799 ± 558.9062 pg/ml, and to Non-Severe Dengue fever is 197.1161 ± 250.8504 pg/ml. IL-6 levels at Day 1 and Day 4 show statistical significance on comparison with the severity of dengue fever, with P-value of 0.0176 on the Day of admission and P-value of 0.0025 on Day 4 of admission.

Table 6: Correlation between IL-6 levels at Day1 of admission and Platelet count at different days of admission

IL-6 levels Day1 vs Platelet Count	p-value	rho
Day1	0.8451	0.0258
Day2	0.2529	-0.1539
Day3	0.0596	-0.2556
Day4	0.0070*	-0.3631
Day5	0.2278	-0.1977
Day6	0.1328	-0.3027
Day7	0.3436	-0.2857

There exists a low negative correlation between IL-6 levels on the Day of admission and Platelet count at day2, day3, day4, day5, day6, day7 of admission which concludes that with increase in the IL-6 levels on the Day of admission there is decrease in the platelet count and vice versa. The correlation value between IL-6 levels on the Day of admission and Platelet count on day 4 of admission is statistically significant, p value being 0.0070.

The same relationship is represented in the Figures 12,13,14,15.

Note: In the figures, rho is represented as R and p-value as p. And the digits were rounded to 2 decimals.

Figure 15: Correlation between IL-6 levels on Day 1 of admission with Platelet count on Day 1 and Day 2 of admission

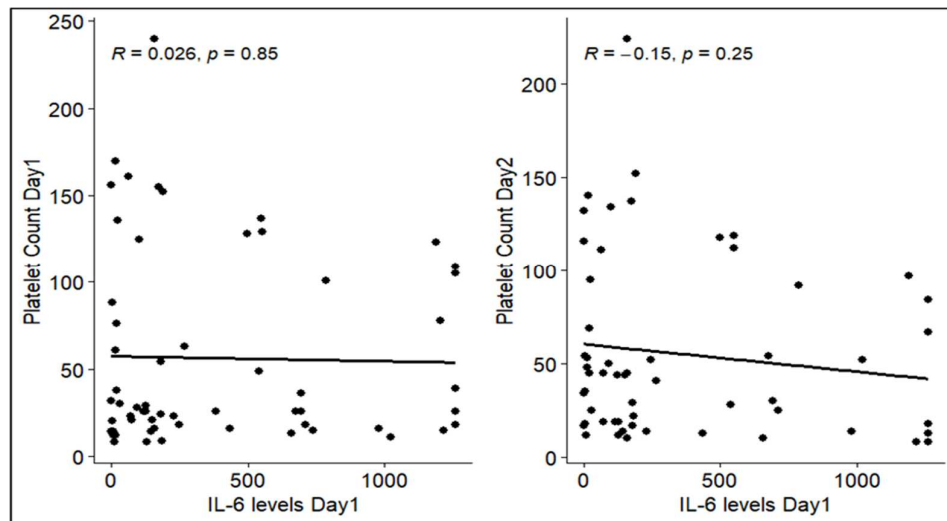


Figure 16: Correlation between IL-6 levels on Day 1 of admission with Platelet count on Day 3 and Day 4 of admission

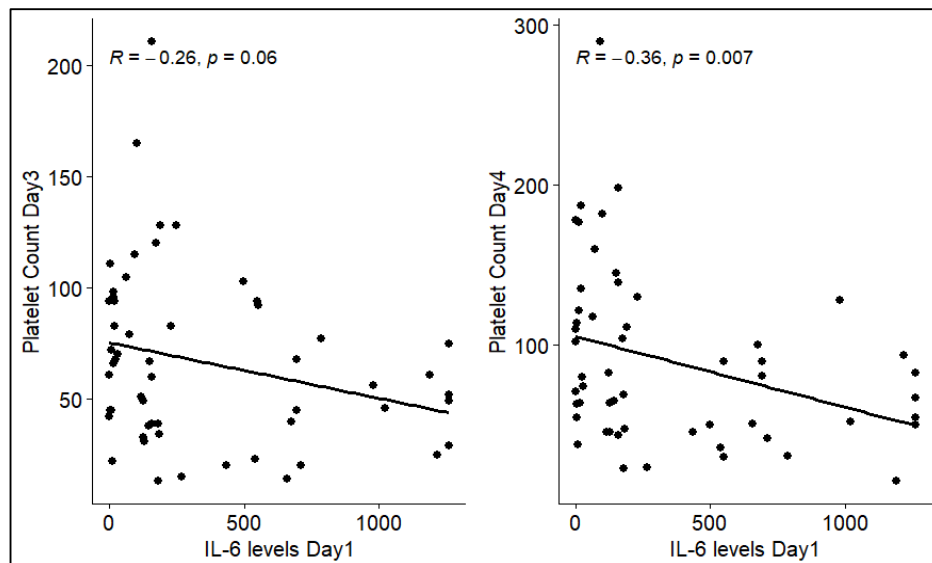


Figure 17: Correlation between IL-6 levels on Day 1 of admission with Platelet count on Day 5 and Day 6 of admission

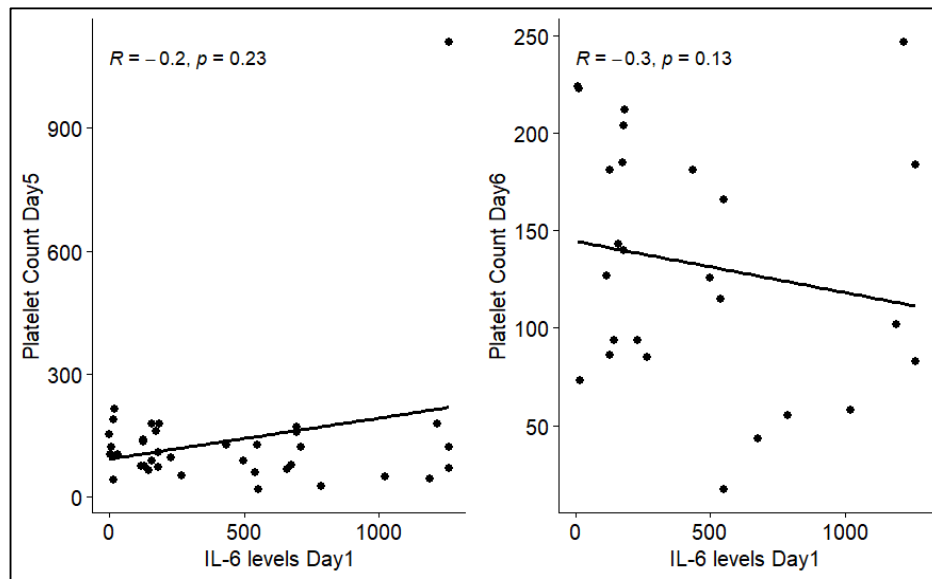


Figure 18: Correlation between IL-6 levels on Day 1 of admission with Platelet count on Day 7 admission

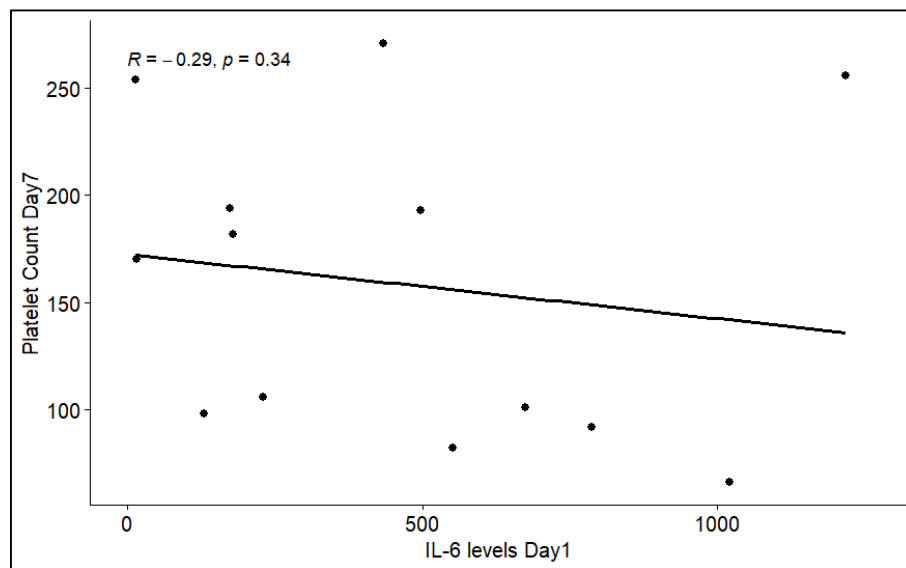


Table 7: Correlation between IL-6 levels at Day 4 of admission and Platelet count at different days of admission.

IL-6 levels Day4 vs	p-value	rho
Platelet Count		
Day1	0.6692	-0.0595
Day2	0.2546	-0.1578
Day3	0.0034*	-0.3916
Day4	0.0391*	-0.2816
Day5	0.5661	-0.0948
Day6	0.5310	-0.1287
Day7	0.5916	0.1644

There exists a low negative correlation between IL-6 levels on Day 4 of admission and Platelet count at day1, day2, day3, day4, day5, day6 which concludes that with decrease in the IL-6 levels there is increase in platelet count and vice versa. The correlation value of IL-6 levels on Day 4 of admission and Platelet count on day 3 and day 4 of admission is significant, p value being 0.0034 and 0.0391 respectively.

The same relationship is represented in the Figures 16,17,18,19.

Note: In the figures, rho is represented as R and p-value as p. And the digits were rounded to 2 decimals.

Figure 19: Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day 1 and Day 2 of admission

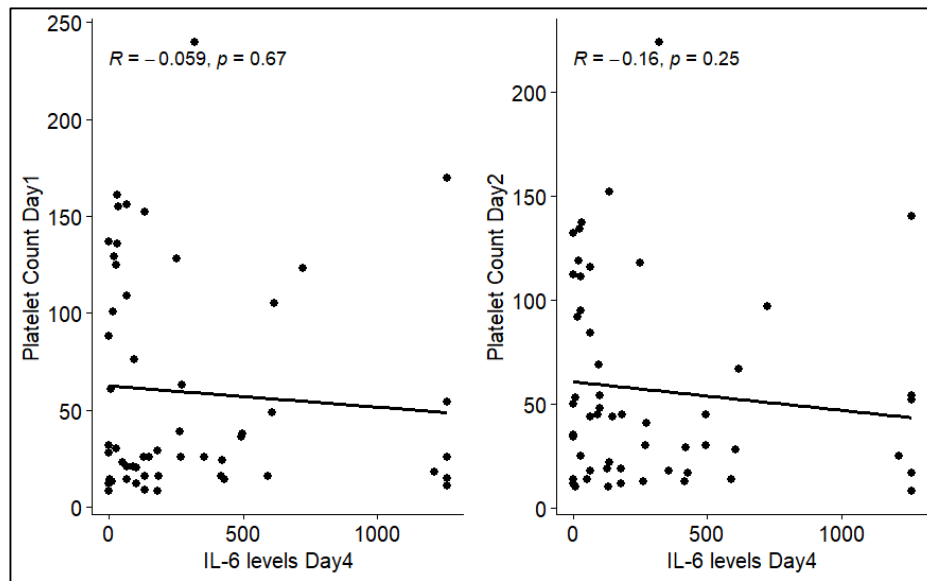


Figure 20: Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day 3 and Day 4 of admission

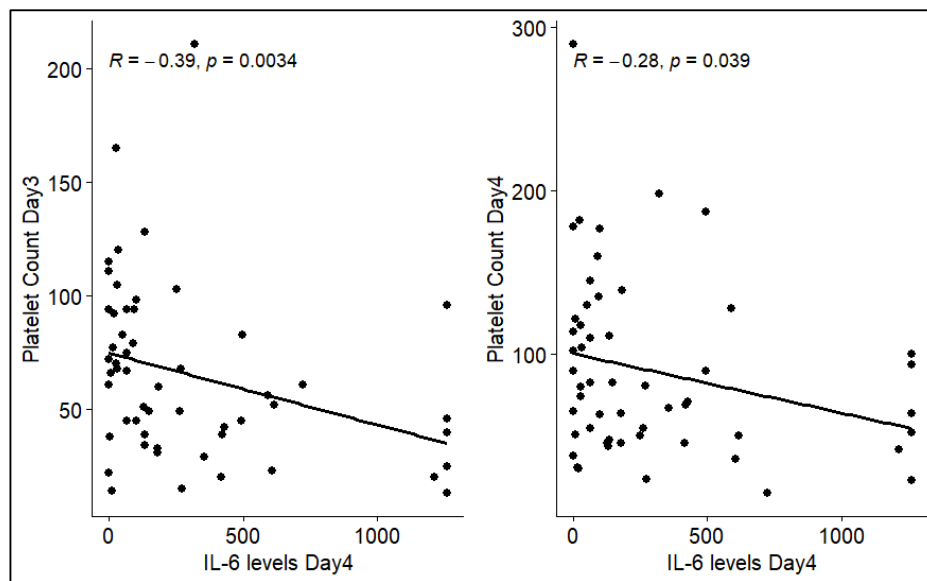


Figure 21: Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day 5 and Day 6 of admission

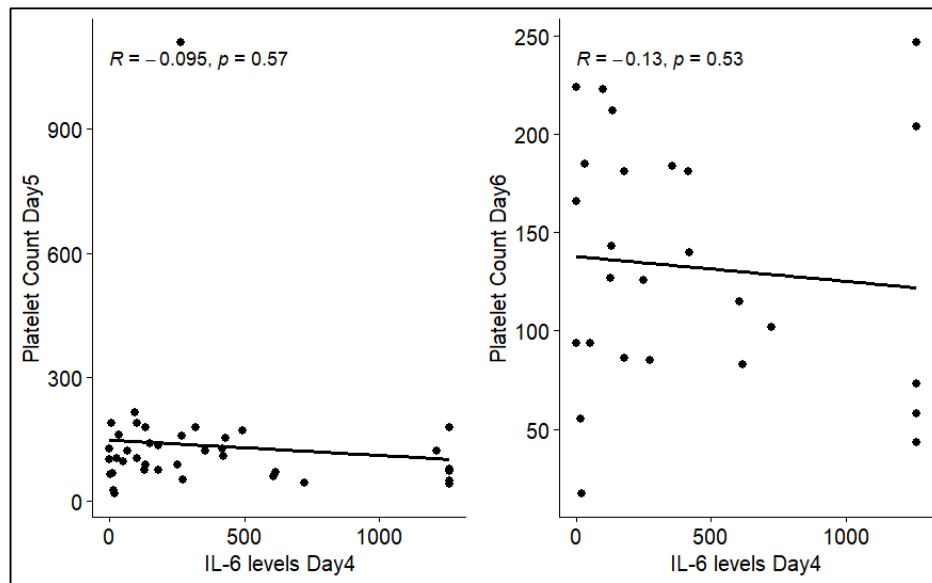


Figure 22: Correlation between IL-6 levels on Day 4 of admission with Platelet count on Day 7 of admission

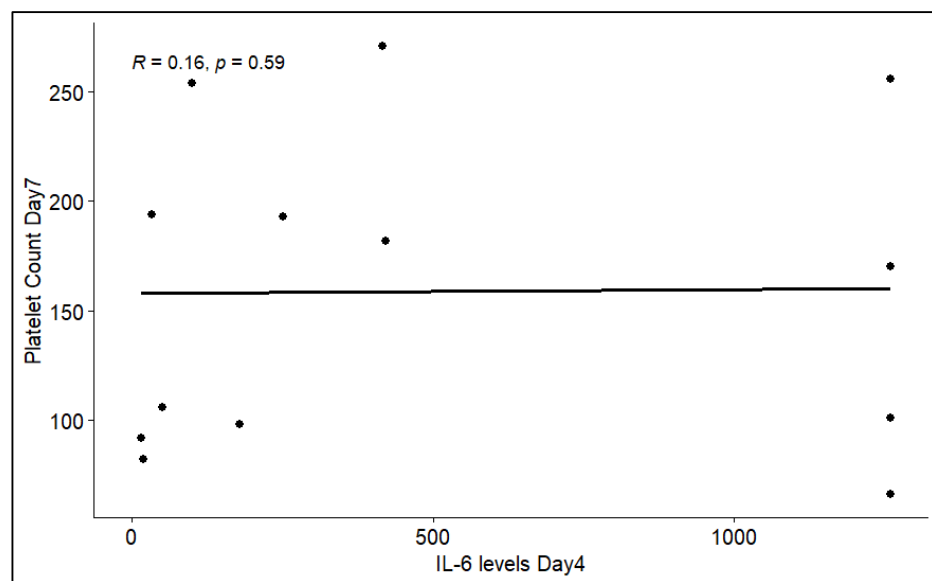


Table 8: Comparison of IL-6 Levels with Outcome of dengue fever

	Outcome		Expired		p-value
			No	Yes	
IL-6 levels (pg/ml)	Day 1 of admission	Mean±SD	340.9873±410.4952	694.5602±439.6781	0.0244*
		Median (Min, Max)	157.4123 (0.0000, 1260)	705.5595(69.999, 1260)	
	Day 4 of admission	Mean±SD	274.8972±369.0034	925.6268±472.8752	0.0571
		Median (Min, Max)	130.7378(0.0000, 1260)	925.6268(591.2535, 1260)	

By Mann-Whitney test, there is significant difference in the distribution of IL-6 Levels at Day1 of admission between the groups. IL-6 levels of 694.5602±439.6781 pg/ml (Mean±SD) on the Day of admission of dengue fever when compared with outcome (expired) showed statistical significance with p value of 0.0244.

Table 9: Comparison of IL-6 Levels with Hospital Stay

	IL-6 levels	p-value	rho
Hospital Stay (Days) vs	Day1of admission (388.83±429.53)	0.3757	0.1164
	Day4of admission (304.63±389.58)	0.0245*	0.3059
p value between day1 and day 4 IL-6 mean level = 0.650			

Spearman correlation (rho) was used to check the correlation between IL-6 Levels and days of hospitalization.

There exists a moderate correlation between hospital stay and IL-6 levels of 304.63±389.58 pg/ml on day 4 of admission which is significant with p value of 0.0245.

The same relationship is represented in the below figures. The relationship between hospital stay and IL-6 levels at day 4 of admission is found to be Linear in nature.

Figure 23: Relationship between Hospital Stay and IL-6 levels on Day 1 and Day 4 of admission

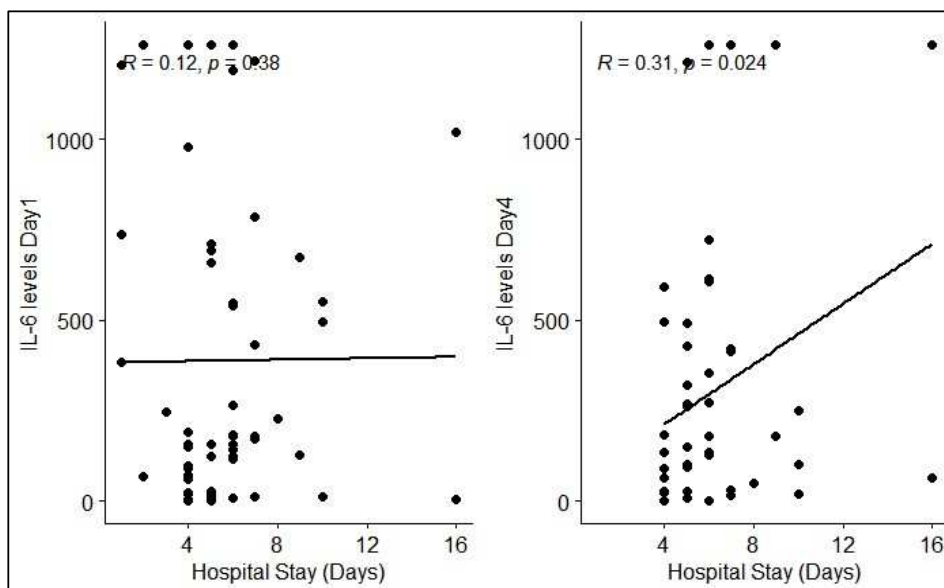


Table 10: Comparison of IL-6 Levels with Inotropic Support

		Inotropic Support	Yes	No	p-value
IL-6 levels (pg/ml)	Day 1 of admission	Mean±SD	536.6495 ± 441.2462	319.3044 ± 409.1429	0.0261*
		Median	496.7625	150.2730	
		(Min, Max)	(13.97, 1260)	(0.00, 1260)	
	Day 4 of admission	Mean±SD	579.5594 ± 557.4219	210.0398 ± 269.5879	0.0178*
		Median	262.6830	94.4385 (0.0000,	
		(Min, Max)	(19.5555, 1260)	1260)	

By Mann-Whitney test, there is significant difference in the distribution of IL-6 Levels at Day1 and Day 4 of admission between the groups. On the day of admission IL-6 levels of 536.6495 ± 441.2462 pg/ml (Mean±SD) showed statistical correlation with the requirement of inotropic support, p value being 0.0261. Similarly on Day 4 of admission IL-6 levels of 579.5594 ± 557.4219 pg/ml (Mean±SD) showed statistical correlation with the requirement of inotropic support, p value being 0.0178.

Table 11: Comparison of IL-6 Levels with RDP Transfusion

		RDP Transfusion	Yes	No	p-value
IL-6 levels (pg/ml)	Day 1 of admission	Mean±SD	407.9806 ± 415.8745	374.8968 ± 441.4313	0.6025
		Median (Min, Max)	180.8325 (0, 1260)	166.0822 (0,1260)	
	Day 4 of admission	Mean±SD	409.7032±481.2795	233.8777±311.0695	0.4355
		Median (Min, Max)	133.7220(0, 1260)	114.4875(0, 1260)	

By Mann-Whitney test, it is found that there is no significant difference in the distribution of IL-6 Levels at Day1 & Day 4 of admission between the groups.

Table 12: Comparison of IL-6 Levels with SDP Transfusion

		SDP Transfusion	Yes	No	p-value
IL-6 levels (pg/ml)	Day 1 of admission	Mean±SD	235.9712 ± 370.3795	414.3759 ± 433.4821	0.1662
		Median (Min, Max)	121.5090 (0, 1260)	182.8455 (0, 1260)	
	Day 4 of admission	Mean±SD	233.7228 ± 363.1452	309.1357 ± 399.0275	0.6686
		Median (Min, Max)	128.6775(0, 1260)	134.5913(0, 1260)	

By Mann-Whitney test, it is found that there is no significant difference in the distribution of IL-6 Levels at Day1 & Day 4 of admission between the groups.

Table 13: Diagnostic test results

		Cut point	Sensitivity	Specificity	NPV	PPV
IL-6 levels Day1 of admission (pg/ml)	Severity of dengue fever at Day1 of admission	>189.7605	68.89 %	80.00%	46.15%	91.17%
IL-6 levels Day4 of admission (pg/ml)	Severity of dengue fever at Day4 of admission	>183.0660	68.18%	70.00%	33.33%	90.90%
IL-6 levels Day1 of admission	Outcome (Expired)	>265.4565	67.30%	75.00%	26.09%	94.59%
IL-6 levels Day4of admission	Outcome (Expired)	>495.7575	84.62%	100.00%	20.00%	100.00%
IL-6 levels Day1 of admission	Outcome (Survived)	<265.4565	94.44%	25.0%	75.00%	65.38%
IL-6 levels Day4 of admission	Outcome (Survived)	<495.7575	100.00%	18.18%	100.00%	80.85%

From the diagnostic test of IL-6 levels day 1 of admission and severity of dengue fever at day1 of admission, cut point of >189.7605 pg/ml is observed in predicting the severity of dengue in patients with the sensitivity of 68.89% and specificity of 80.00%, NPV of 46.15% and PPV of 91.17%. For IL-6 levels on day 4 of admission and severity of dengue fever at day 4of admission, cut point of >183.0660 pg/ml is observed in predicting the severity of dengue in patients with the sensitivity of 68.18% and specificity of 70.00%, NPV of 33.33% and PPV of 90.90%.

From the diagnostic test of IL-6 levels on day1 of admission and outcome, cut point of >265.4565 is observed in predicting mortality with the sensitivity of 67.30%, specificity of 75.00%, NPV of 26.09% and PPV of 94.59%. For IL-6 levels on day4 of admission and outcome, cut point of >495.7575 is observed in predicting mortality with the sensitivity of 84.62%, specificity of 100.00%, NPV of 20.00% and PPV of 100%.

Figure 24: ROC curve for the diagnostic test of IL-6 levels Day1 of admission and Severity of dengue fever at Day1 of admission

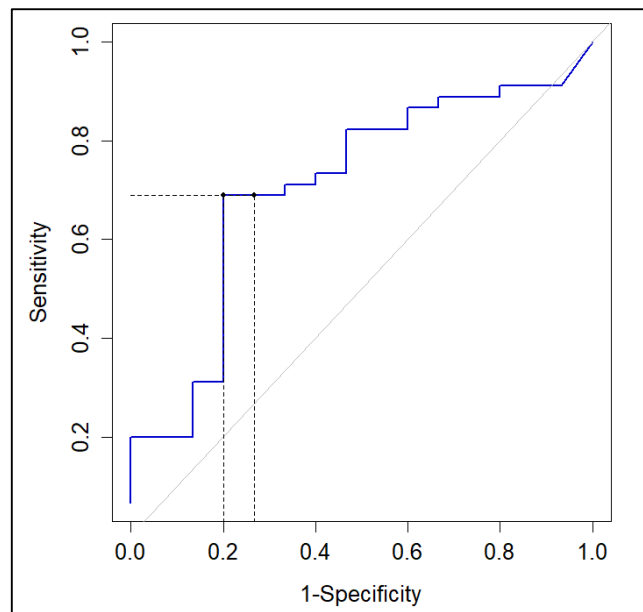


Figure 25: ROC curve for the diagnostic test of IL-6 levels Day4 of admission and Severity of dengue fever at Day4 of admission

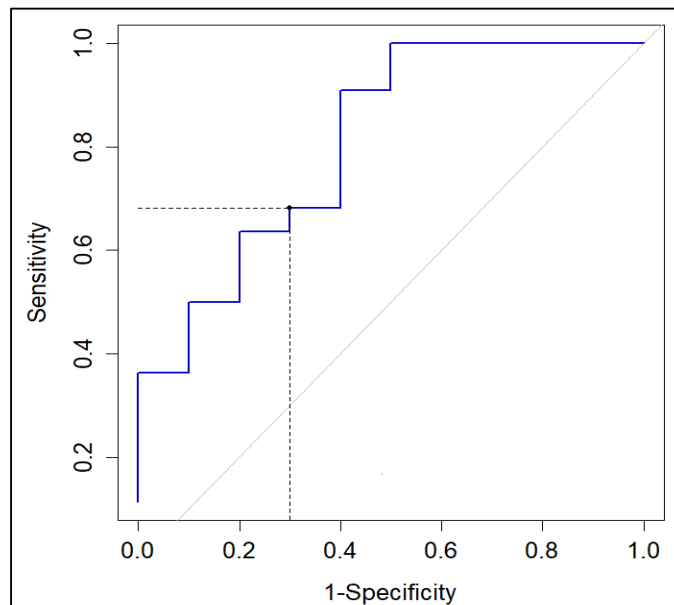


Figure 26: ROC curve for the diagnostic test of IL-6 levels Day1 of admission and Outcome (Expired)

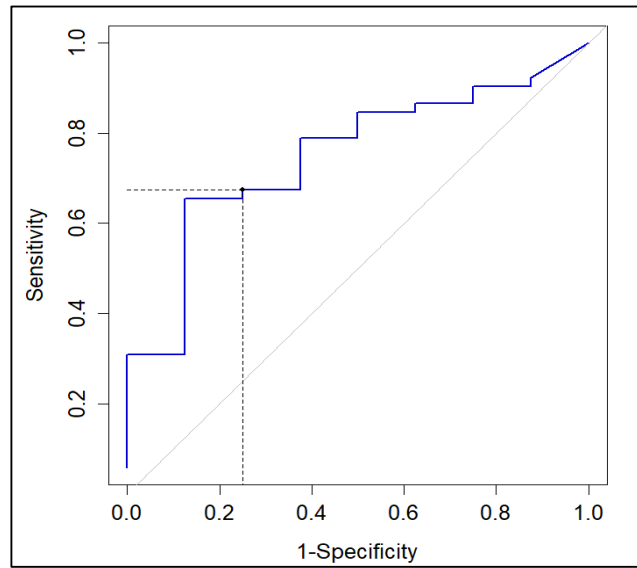
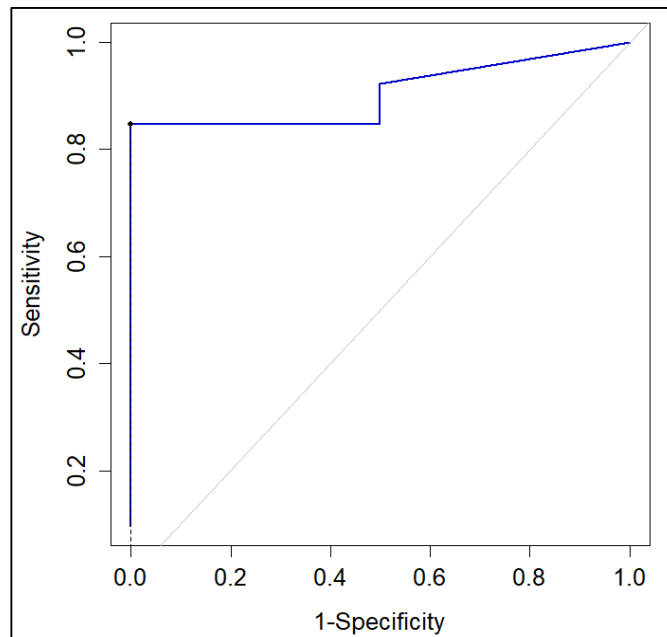


Figure 27: ROC curve for the diagnostic test of IL-6 levels Day4 of admission and Outcome (Expired)



DISCUSSION

Dengue Fever is self-limiting fever that typically lasts 5-7 days. DF can be debilitating during acute sickness stage. Clinical characteristics of DF differ depending on the patient's age. Infants/Young children experience undifferentiated febrile illness with a maculopapular rash. Adults and older children may have moderate febrile illness or severe illness with high fever (typically biphasic), nausea, vomiting, retroorbital discomfort, severe headache, myalgia, arthralgia, and petechiae. Thrombocytopenia and leukopenia can occur at any age. In certain circumstances, DF may be associated with a bleeding complication like epistaxis, hematuria, gastrointestinal bleeding, gingival bleeding, or menorrhagia.⁸

While immune response activation, particularly cytokine activation, is required to regulate viral replication, an excess of cytokines leads to increased inflammation and disease development. DENV has the ability to alter host systems for its own survival, such as switching protective Th1 responses to anti-inflammatory Th2 responses, resulting in a more severe hemorrhagic infection. Increased production of anti-inflammatory cytokines including IL-6 and IL-10 promotes Dengue virus proliferation and illness development. Previous studies have found an elevated profile of pro-inflammatory cytokines in individuals with Dengue fever and Dengue hemorrhagic fever or Dengue with warning symptoms; however, evidence for severe Dengue is limited.^{9,48,49}

This present study aimed to assess the IL-6 levels in predicting the severity of dengue fever.

Present study included total of 60 patients fulfilling inclusion criteria, among them 40% were female and 60% were male dengue positive patients, with male preponderance. On assessment of Age wise distribution, we identified a higher prevalence of dengue positive among the children in age of 11-15yrs (38.3%) followed by 6-10yrs (26.7%).

In a study by Suharti C et al., the mean age of participant was 6.6yrs (SD 2.8), with 52% female child and 48% male child, with nearly equal distribution between the genders.³⁴ In study by Imad H et al., 38% were female patients and 62% were male patients with male preponderance in the study.⁴⁷ In study by Kanwal F et al., 76.7% of patients were female and 23.3% were male patients, with female preponderance in the study.⁵⁰

On Day 1 of admission out of 60 patients, 45(75%) belonged to Non- Severe Dengue category and 15(25%) belonged to Severe Dengue. Similarly, on Day 4 of admission out of 60 patients, 44(73.33%) belonged to Non- Severe Dengue category and 10(16.67%) belonged to Severe Dengue and 6(10%) expired till Day 4 of admission. In present study out of 60 patients who got admitted with dengue fever, on Day 1 of admission, 18 patients had Day 4 of illness out of which 10 (55.56%) patients had Severe Dengue. By using Monte- Carlo's simulation in chi-square test, there is significant association present between day of illness on Day 1 of admission with severity of dengue fever, p value being 0.003498. Also, 30% of the patients who presented to hospital on the Day of admission had Day 4 of illness and in that 55.56% had Severe Dengue.

On assessment of IL-6, there is significant difference in the distribution of IL-6 Levels at Day1 and Day 4 between the groups. IL-6 levels at Day 1 and Day 4 show statistical significance on comparison with the severity of dengue fever, with p-value of 0.0176 on Day 1 of admission and p-value of 0.0025 on Day 4 of admission. A moderate correlation is also present between hospital stay and IL-6 levels at day 4 of admission which is significant with p value of 0.0245. Similarly, there exists a low negative correlation between IL-6 levels at Day 1 of admission and Platelet count at day2, day3, day4, day5, day6, day7 of admission and low positive correlation between IL-6 levels at Day 1 of admission and Platelet count at Day 1 of admission. The correlation value of IL-6 levels at day 1 of admission and Platelet count at day 4 of admission is significant. Also, we found that IL-6 levels on Day 1 and Day 4 of admission had significant correlation with requirement of inotropic support in dengue patients. And the IL-6 levels on Day 4 of admission had statistical significance with the duration of hospital stay.

In continuation, we also documented that the day 4 mean levels were higher than the day 1 mean levels among the patients had the requirement of inotropes, longer duration stay and death of patients.

In study by Sehrawat P et al., similar to present study found a significant higher median level of IL-6 among the patients with severe dengue compared to the patients with non-severe dengue.⁵¹ Recent research has focused on the function of cytokines and other biomarkers in the pathophysiology of severe dengue and the usefulness of these biomarkers as risk factors.^{52,53} The inflammatory response associated with dysregulated cytokine production has been convincingly proven to play essential roles in the development of severe dengue.⁵⁴ In numerous

investigations, greater levels of cytokines such as TNF- Alpha, IL-1, IFN-, IL-4, IL-6, IL-13, IL-7, and GM-CSF were linked to severe dengue fever.^{51,55-57} Because IL-6 levels are increased in severe dengue, they are strong indicators of dengue severity. IL-6 estimate at presentation can help us determine if a patient is likely to develop severe dengue signs or not. In study by Bethell D et al., there is significant higher IL-6 with the severity of disease and also with outcome among the patients.⁵²

In a study by Priyadarshini D et al., Mean levels of Interleukin-6 in the controls (healthy individuals) was 4.8 pg/ml and the cut off value was 8.9 pg/ml. However, in dengue infected cases values ranged from 0.13 to 417 pg/ml. The levels of Interleukin-6 in the cases were higher than the controls, but they were not statistically significant ($p>0.05$). Though, after the disease categorization, IL-6 levels in Dengue Hemorrhagic Fever were higher than in Dengue Fever patients ($p=0.02$, Tukey test). Dengue Hemorrhagic Fever cases showed higher mean values also a large group of cases with high levels (47%).⁵⁵

In study by Kanwal F et al., similar to present study documented mean levels of Interleukin-6 expression in healthy subjects as 6.72pg/ml, while in Dengue Fever and Dengue Hemorrhagic Fever groups mean level of Interleukin-6 expression increased up to 150 and 591.59 pg/ml, respectively. The levels of Interleukin-6 expression was significantly lower in DF group than that in DHF group.⁵⁰ In study by Varghese A et al., the IL-6 was positively correlated with severity of the disease and also with the level of liver enzyme markers like serum glutamic oxaloacetic transaminase. IL-6 and TNF alpha levels were considerably high (p value of <0.05) in Severe Dengue. However, there was no significant correlation of Interferon Gamma (p value of >0.05) between control and infected subjects, despite the fact that it was

higher in the infected group. All three cytokines were shown to have a negative connection with platelet count.⁴⁶

From the diagnostic test of IL-6 levels day 1 of admission and severity of dengue fever at day1 of admission, cut point of >189.7605 is observed in predicting the severity of dengue in patients with the sensitivity of 68.89% and specificity of 80.00%, NPV of 46.15% and PPV of 91.17%. For IL-6 levels day 4 of admission and severity of dengue fever at day 4of admission, cut point of >183.0660 is observed in predicting the severity of dengue in patients with the sensitivity of 68.18% and specificity of 70.00%, NPV of 33.33% and PPV of 90.90%.

From the diagnostic test of IL-6 levels day 1 of admission and outcome, cut point of >265.4565 is observed in predicting the mortality with the sensitivity of 67.30%, specificity of 75.00%, NPV of 26.09% and PPV of 94.59%. For IL-6 levels day 4 of admission and outcome, cut point of 495.7575 is observed in predicting the mortality with the sensitivity of 84.62%, specificity of 100.00%, NPV of 20.00% and PPV of 100%.

Our findings indicate that variance in IL-6 levels is linked with Dengue severity. As a result, IL-6 can be utilized to predict illness severity. This might help with patient triage because determining the severity of disease at the time of diagnosis can be challenging. Early detection of more advanced illness, along with more proactive therapy, may lead to better results in severe infections.

CONCLUSION

The present study concludes that serum IL-6 is a strong predictor of the severity of dengue fever and also predicts the clinical outcome of the patients. There is significant strong diagnostic accuracy of detecting the patients with severe dengue and also worst outcome among the patients with high IL-6 level at presentation. Also, there is significant higher mean levels of the IL-6 on day 4 with worst outcome, requirement of inotropes and hospital stay compared to the day 1 results. Thus, measurement of IL-6 on the day of admission will help the clinician to identify the severity of dengue infection at admission also assist in planning proper treatment strategy depending on severity. There is requirement of further studies to confirm and also other dependent factors including other cytokines to envisage the mechanism of dengue severity. This will help the clinician to be at caution and also act accordingly with the IL-6 at the time of admission by predicting the expected clinical outcome of patient.

LIMITATIONS AND SCOPE OF THE STUDY

- As it's a single centre study, more multi centered and larger number of studies are required from different geographical areas to make our findings more concrete.
- From our study it is observed that IL-6 levels can be used in early prediction of severe dengue fever and thus help in providing timely and appropriate treatment which might help in decreasing mortality. Therefore, this study recommends the use of IL-6 levels as a marker of severity of dengue fever.
- There is lot of scope in future research to know how the IL-6 levels vary in primary vs secondary dengue. Thus, it will be interesting to know whether IL-6 levels can be used in differentiating between primary and secondary dengue fever.

SUMMARY

The present cross-sectional study was conducted among the patients attending or admitted to the hospital, Department of Pediatrics J N Medical College and Hospital, Belgaum's KAHER Dr Prabhakar Kore Hospital PICU.

This present study aimed to assess the IL-6 levels in predicting the severity of dengue fever.

Present study included total of 60 patients fulfilling inclusion criteria, with male preponderance (60% males).

- In this study, a higher prevalence of dengue positive patients belonged to age of 11-15yrs (38.3%) followed by 6-10yrs (26.7%).
- On Day 1 of admission out of 60 patients, 45(75%) belonged to Non- Severe Dengue category and 15(25%) belonged to Severe Dengue.
- On Day 4 of admission out of 60 patients, 44(73.33%) belonged to Non- Severe Dengue category and 10(16.67%) belonged to Severe Dengue and 6(10%) had expired till Day 4 of admission and total 8 patients expired during the study.
- In present study there is significant association present between days of illness at Day1 of admission with severity of dengue fever, p value being 0.003498.
- IL-6 levels at Day 1 and Day 4 show statistical significance on comparison with the severity of dengue fever, with p-value of 0.0176 on Day 1 of admission and p-value of 0.0025 on Day 4 of admission.
- A negative correlation is also seen between IL-6 levels on Day 1 of admission and platelet count at day2, day3, day4, day5, day6, day7 of admission which

concludes that with increase in the IL-6 levels on the Day of admission there is decrease in the platelet count and vice versa.

- Similarly, A negative correlation is also present between IL-6 levels on Day 4 of admission and at day1, day2, day3, day4, day5, day6 of admission which concludes that with decrease in the IL-6 levels there is increase in platelet count and vice versa.
- A moderate correlation is present between hospital stay and IL-6 levels at day 4 of admission which is statistically significant with p value of 0.0245.
- There is significant difference in the distribution of IL-6 Levels at Day1 and Day 4 of admission between the groups.
- There is statistical correlation between IL-6 levels with inotropic support on Day 1 and Day 4 of admission with p value of 0.0261 and 0.0178 respectively
- On the Day of admission IL-6 cut of levels of >189.76 is observed in predicting the severity of dengue in patients with the sensitivity of 68.89% and specificity of 80.00%, NPV of 46.15% and PPV of 91.17%.
- On Day 4 of admission IL-6 cut of levels of >183.06 is observed in predicting the severity of dengue in patients with the sensitivity of 68.18% and specificity of 70.00%, NPV of 33.33% and PPV of 90.90%.
- From the diagnostic test of IL-6 levels day 1 of admission and outcome, cut point of >265.45 is observed in predicting the mortality with the sensitivity of 67.30%, specificity of 75.00%, NPV of 26.09% and PPV of 94.59%. For IL-6 levels day 4 of admission and outcome, cut point of 495.77 is observed in predicting the mortality with the sensitivity of 84.62%, specificity of 100.00%, NPV of 20.00% and PPV of 100%.

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ANNEXURE I – CONSENT FORM

**K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
J.N. Medical College, Belagavi**

INFORMED CONSENT FORM

“IL-6 LEVELS IN PREDICTION OF SEVERITY OF DENGUE FEVER – ONE YEAR CROSSECTIONAL OBSERVATIONAL STUDY”

Principal Investigator: REG NO. BM0120009

Guide: Dr. _____.

Respected Sir/Madam,

We invite you to participate in our study as you are eligible for the same. During the study, you will be asked some questions in detail regarding your present complaints.

Purpose of the study: There have been multiple studies done so as to formulate the guidelines for correlation of IL-6 levels with severity of dengue fever and platelet count.

The primary purpose of your participation in this is to study IL-6 levels to predict the severity of dengue fever and then correlate the levels of IL-6 with platelet count in dengue.

Risks and Benefits: The result of you taking part in this research would help health care providers towards a better understanding of IL-6 levels in dengue and its correlation with the severity of dengue illness.

Alternatives: If you decide not to participate in this study, you will still be receiving the usual standard of care.

Privacy and confidentiality: Your privacy will be respected, and all information collected about you during this study will be kept confidential. Your identity will remain undisclosed.

Relations with the Institutional policy: The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries because of participating in this project.

Financial incentives: You shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results: The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will always be maintained confidential.

Voluntary participation: Your participation in this study is voluntary. Your decision whether to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. In the event, if you suffer any physical injury as the result of your participation in this study, you may contact the investigator of the study.

In the event of an emergency, you should contact KLE'S Dr. Prabhakar Kore Hospital and MRC on Telephone No. 08312473777.

In case you need further information regarding your rights as a study participant, you may please contact DR. HARSHA HEGDE, Chairperson, JNMC, IEC and Scientist D, ICMR, National Institute of Traditional Medicine.

Legal Rights: By signing this consent form; you are not waiving any of your Legal rights.

Consent statement:

“I volunteer and consent to participate in the study. I have read (or it has been read to me in the language known to me) the information sheet thoroughly. Full opportunity was given to me to ask questions. I am fully satisfied with the answers to the questions I wanted to ask. I hereby voluntarily agree to participate in this research project”.

I have read the information in this form. After understanding all details about the study, I agree to give assent to be included as a volunteer in the study titled **“IL-6 LEVELS IN PREDICTION OF SEVERITY OF DENGUE FEVER – CROSS SECTIONAL OBSERVATIONAL STUDY”**

_____	_____
Name of the Participant	Signature of the participant or Left-Hand Thumb impression
_____	_____
Name of the Parent	Signature of the parent
_____	_____
Name of Investigator	Signature of investigator
_____	_____
Name of Witness	Signature of Witness
Date: _____	Place: _____

Queries

If you have any queries, you may contact

REG NO. BM0120009

Post Graduate Student

Department Of Paediatrics

JNMC, Belagavi-590010

DR. _____.

Professor and Head

Department of Paediatrics,

KLE Academy of Higher Education & Research,

Jawaharlal Nehru Medical College,

Belgaum -590010

If you have any questions about your rights or research participation you may contact

Chairman ethical committee:

DR. HARSHA HEGDE

Chairperson, Jawaharlal Nehru Medical College,

IEC And Scientist D,

ICMR, National Institute of Traditional Medicine

Belagavi- 590010

Phone-9480422500

ANNEXURE II – PROFORMA

**“IL-6 LEVELS IN PREDICTION OF SEVERITY OF DENGUE
FEVER - CROSSECTIONAL OBSERVATIONAL STUDY”**

Overall Serial No:

IP Number:

Date:

Sociodemographic Particulars

Informant's Name

Relation

Profession

Education

Permanent Residence:

Contact Telephone:

Patient's Name:

Gender: Male/Female

Date of Admission:

Age:

Dengue ± warning signs



Severe dengue



Criteria for dengue ± warning signs

Probable dengue

- Live in/travel to dengue endemic area. Fever and 2 of the following criteria:
- Nausea, vomiting
 - Rash
 - Aches and pains
 - Tourniquet test positive
 - Leucopenia
 - Any warning sign

Laboratory confirmed dengue

(important when no sign of plasma leakage)

Warning signs*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2cm
- *Laboratory:* Increase in HCT concurrent with rapid decrease in platelet count

* Requiring strict observation and medical intervention

Criteria for severe dengue

1. Severe plasma leakage, leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

2. Severe bleeding as evaluated by clinician

3. Severe organ involvement

- Liver: AST or ALT >= 1000
- CNS: Impaired consciousness
- Heart and other organs

IL-6 levels

Day-1 of Admission	Day-4 of Admission

Classified as per WHO criteria-

	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Nausea/vomiting							
Abdominal Pain							
Mucosal Bleed							
Decrease in Platelet Count							
Liver Enlargement							
BP(SBP/DBP)							
Organ involvement							
Classification as per WHO criteria							

Platelet And PCV Count

	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
Platelet Count							
PCV							

SEVERITY ASSESSMENT

	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
PR (beats/min)							
SBP (mm Hg)							
DBP (mm Hg)							
RR (breaths/min)							
Urine Output (ml/kg/hr)							
Temperature (Degree F)							

(Signature of Investigator)

Sl. No.	Age(years)	Gender	Dengue			Day of Illness on day 1 of Admission	Day of Illness on day 4 of Admission	Hospital Stay (Days)	IL-6 levels		Nausea/Vomiting							Abdominal Pain							Mucosal Bleed							Decrease in Platelet Count							Liver Enlargement							
			NSI	IgM	IgG				Day1	Day4	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	
1	14	Male	Positive	Positive	Positive	5	8	6	>1260	354.783	Y	N	N	N	N	N		Y	Y	Y	Y	N	N		N	N	N	N	N	N		Y	Y	N	N	N	N		N	N	N	N	N	N		
2	15	Male	Negative	Positive	Positive	2	5	6	125.8995	179.79	Y	N	N	N	N	N		N	N	Y	N	N	N		Y	N	Y	N	N	N		Y	Y	N	N	N	N		N	N	N	N	N	N		
3	17	Male	Negative	Positive	Negative	3	6	7	179.2095	421.014	Y	N	Y	Y	N	N	N	Y	Y	N	N	N	N		N	N	N	N	N	N		Y	Y	N	N	N	N		N	N	N	N	N	N		
4	6	Male	Positive	Negative	Negative	4	7	5	156.8925	320.2335	Y	N	Y	N	N	N		Y	Y	N	Y	N		N	N	N	N	N		Y	Y	Y	Y	Y		N	N	N	N	N	N					
5	12	Male	Negative	Positive	Positive	2	5	7	173.976	33.159	Y	N	N	N	N	N	N	Y	Y	Y	N	N	N		N	N	N	N	N		Y	Y	Y	Y	N	N		N	N	N	N	N	N			
6	10	Male	Positive	Positive	Positive	5	8	9	129.033	179.7645	Y	N	N	N	N	N	N	Y	Y	Y	N	N	N		N	N	N	N	N		Y	N	N	N	N	N		Y	Y	Y	Y	Y	Y			
7	5	Male	Positive	Positive	Positive	4	7	5	19.869	94.4385	Y	N	N	N	N			Y	Y	Y	Y	N		Y	N	N	N	N		Y	Y	N	N	N		N	N	N	N	N						
8	17	Male	Positive	Positive	Positive	3	6	6	117.1185	128.6775	Y	Y	Y	N	N	N		Y	Y	Y	N	N	N		Y	N	N	N	N		Y	Y	N	Y	N	N		Y	Y	Y	Y	N	N			
9	14	Male	Negative	Positive	Positive	1	4	6	10.3125	0	Y	Y	N	N	N	N		Y	Y	Y	Y	N	N		N	N	N	N	N		Y	N	N	N	N	N		Y	Y	Y	Y	N	N			
10	8	Female	Negative	Positive	Positive	2	5	4	0	0	Y	N	N	N				Y	N	N	N			N	N	N	N		Y	N	N	N				N	N	N	N							
11	5	Male	Positive	Positive	Positive	4		1	735.9015		Y							Y						N						Y							Y									
12	11	Male	Positive	Positive	Positive	2	5	4	19.2945	495.7575	Y	N	N	N				Y	Y	Y	N			N	N	N	N		Y	N	N	N				Y	Y	Y	Y							
13	9months	Male	Positive	Negative	Negative	4	7	6	539.2995	606.819	Y	Y	N	N	N	N		Y	Y	N	N	N		N	Y	Y	N	N	N		Y	Y	Y	N	N	N		Y	Y	Y	Y	Y	Y			
14	15	Male	Positive	Positive	Positive	5	8	7	433.9395	415.977	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	N	N	N		Y	Y	N	N	N	N		Y	Y	Y	Y	Y	Y	N		
15	9	Male	Positive	Negative	Negative	2	5	10	13.97	100.4	Y	Y	Y	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N		Y	N	N	N	N	N		Y	Y	Y	Y	Y	Y	Y		
16	10	Female	Negative	Positive	Negative	3	6	6	143.523	1.3305	Y	N	N	N	N	N		Y	N	N	N	N	N		N	N	N	N	N		Y	Y	N	N	N	N		Y	Y	Y	N	N	N			
17	14	Male	Positive	Positive	Positive	3	6	6	182.8455	134.646	N	N	N	N	N	N		Y	Y	Y	Y	Y	N	N	N	N	N	N		Y	N	N	N	N	N		N	N	N	N	N	N				
18	14	Male	Positive	Negative	Negative	5	8	6	549.792	0.2865	Y	Y	N	N	N	N		N	N	N	N	N		N	N	N	N	N		Y	Y	Y	Y	N	N		N	N	N	N	N					
19	11	Female	Positive	Positive	Positive	4	7	16	1021.218	>1260	Y	Y	Y	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N		Y	N	Y	N	Y	N	N	Y	Y	Y	Y	Y	Y	Y		
20	10	Male	Negative	Positive	Positive	4		1	382.5585		Y							Y						N						Y							Y									
21	13	Male	Positive	Negative	Negative	5		3	246.4485		N	N	N					Y	Y	Y				N	N	N				Y	N	N					Y	Y	Y							
22	4	Female	Positive	Negative	Negative	4		2	69.999		Y	Y						Y	Y					N	N					Y	Y						Y	Y								
23	7months	Male	Positive	Negative	Negative	2	5	5	3.36	101.3805	N	N	N	N	N			N	N	N	N	N		Y	Y	Y	Y	N		Y	N	Y	N	N		N	N	N	N	N						
24	4	Female	Positive	Negative	Positive	5	8	4	980.4975	591.2535	N	N	N	N				Y	Y	N	N			N	N	N	N		Y	Y	N	N				Y	Y	Y	Y							
25	14	Male	Positive	Negative	Negative	1	4	4	1.6145	0	N	N	N	N				N	N	N	N			N	N	N	N		Y	N	Y	N				N	N	N	N							
26	15	Female	Negative	Positive	Negative	5	8	10	496.7625	251.3565	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y	Y	N	N	Y	Y	N	N	N		Y	Y	Y	Y	N	N		Y	Y	Y	Y	Y	Y	Y		
27	16	Female	Negative	Positive	Positive	2	5	16	4.947	65.2185	N	N	N	N	N			Y	Y	N	N	N		N	N	N	N		Y	N	N	N	N			N	N	N	N							
28	16	Male	Negative	Positive	Positive	2	5	4	6.0585	0	N	N	N	N				N	N	N	N			N	N	N	N		Y	N	N	N				N	N	N	N							
29	6	Male	Negative	Positive	Positive	4	7	8	229.2165	50.025	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	N	N	N	N	N	N		Y	Y	N	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N		
30	10	Female	Positive	Positive	Positive	6	9	6	265.4565	272.898	Y	Y	N	N	N	N		Y	Y	Y	N	N	N		N	N	N	N	N		Y	Y	Y	N	N	N		N	N	N	N	N				
31	14	Male	Negative	Positive	Positive	2	5	5	0	428.004	N	N	N	N	N			Y	Y	Y	N	N		Y	Y	N	N	N		Y	N	N	N				N	N	N	N						
32	10	Female	Positive	Negative	Negative	3	6	6	178.8195	>1260	Y	N	N	N	N	N		Y	Y	Y	N	N	N		N	N	N	N	N		Y	Y	Y	N	N	N		Y	Y	Y	Y	N	N			
33	6	Female	Positive	Positive	Negative	4	7	10	552.012	19.5555	Y	Y	N	N	N	N	N	N	Y	Y	N	N	N	N	Y	N	N	N	N		Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	N			
34	16	Female	Positive	Positive	Positive	3	6	6	1189.1655	722.1165	N	N	N	N	N	N		N	N	N	N	N		N	N	N	N	N		Y	Y	Y	Y	N	N		N	N	N	N	N					
35	6	Female	Positive	Positive	Negative	4	7	7	786.7365	14.8815	Y	N	N	N	N	N	N	Y	Y	Y	Y	N	N	N	N	N	N	N		Y	Y	Y	Y	Y	N	N	N	N	N	N	N					
36	14	Male	Negative	Positive	Positive	3	6	5	693.708	493.1415	N	N	N	N	N			N	N	N	N	N		N	N	N	N		Y	Y	N	N	N			N	N	N	N							
37	10	Male	Negative	Positive	Positive	2	5	4	62.637	28.449	N	N	N	N				N	N	N	N			N	N	N	N		Y	Y	Y	N				N	N	N								
38	13	Female	Positive	Negative	Negative	5		2	>1260		Y	N						N	N					N	N				Y	Y						N										
39	16	Female	Positive	Positive	Negative	2	5	5	123.9915	148.398	Y	N	N	N	N			Y	Y	Y	Y	N		N	N	N	N		Y	N	N	N	N			N	N	N	N							
40	7	Female	Negative	Positive	Positive	4		1	1205.859		N																																			

BP (SBP/DBP)							Pleural Effusion							Carditis/Pericardial Effusion							Encephalitis							Classification as per WHO criteria			
Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4
90/60	88/52	92/60	94/60	94/62	112/78		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
98/62	104/70	92/60	96/60	108/68	110/70		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
110/90	120/70	110/70	100/70	110/70	110/70	104/78	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
98/64	108/62	110/60	104/62	100/60			N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
102/60	100/58	100/60	100/60	110/70	110/62	106/70	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
90/70	110/70	110/60	96/64	98/64	100/68	110/72	N	N	N	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	DSS
108/64	104/60	94/60	98/62	90/60			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
110/70	120/84	118/78	120/80	122/78	120/78		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
104/70	114/78	114/80	116/78	110/70	108/70		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
96/70	102/68	98/60	98/64				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
70/40							N							N							Y							DSS			
90/60	94/60	92/60	98/66				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
96/62	102/70	100/62	98/78	110/60	94/60		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
100/60	112/74	108/70	120/7-	122/76	124/74	102/70	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
84/60	92/60	94/60	90/60	88/62	90/64	98/70	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
100/60	96/70	92/70	90/68	100/64	110/68		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
102/64	106/68	110/62	118/66	124/80	104/64		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
112/78	116/72	102/68	116/70	112/70	110/70		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
98/64	88/54	82/60	80/52	88/54	92/64	90/60	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
80/60							Y							Y							Y							DSS			
80/58	90/62	102/64					Y	Y	Y					Y	Y	Y					N	N	N					DSS	DSS	DSS	
94/66	102/68						Y	Y						Y	Y						N	N						DSS	DSS		
98/58	86/50	92/58	90/60	94/62			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
108/70	104/72	106/80	102/76				N	Y	Y	Y				Y	Y	Y	N				N	N	N	N				DSS	DSS	DSS	DSS
98/58	100/60	110/70	108/70				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
90/40	90/48	94/50	106/60	92/60	120/74	94/50	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
100/72	126/72	116/74	130/90	120/80			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
130/90	100/60	110/60	110/70				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
90/62	130/90	126/88	120/80	126/90	100/60	98/60	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
92/60	100/70	100/60	98/60	100/60	110/60		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
104/76	112/68	118/76	102/68	110/68			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
98/60	98/62	84/60	82/60	98/68	100/68		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	DSS	DSS
108/60	110/60	94/60	106/68	100/82	120/80	92/60	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
120/74	124/80	108/64	110/80	110/78	112/78		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
88/62	90/60	88/60	84/60	90/60	110/68	98/60	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
110/80	112/78	114/76	118/80	120/80			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
110/70	108/68	108/70	110/72				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
100/70	60/30						N	N						Y	Y						Y	Y						DSS	DSS		
98/62	100/68	114/72	114/70	112/70			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
90/68							N							N							Y							DSS			
108/70	106/70	108/70	106/82	110/88	102/70	108/70	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
110/70	116/72	114/70	120/80				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
100/70	110/70	90/60	96/60	94/62			N	N	N	N	N			N	N	N	N	N			N	N	N	N	N			Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
106/70	106/72	108/74	108/60				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
100/60	100/64	104/60	104/70				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
104/58	102/60	104/62	100/60				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs
110/72	112/70	108/70	122/64				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
110/70	106/70	110/70	122/64	128/70	120/70		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
110/70	110/72	128/70	120/8-				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
126/80	120/74	120/70	124/70				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs
90/50	120/90	118/62	110/70	116/70	116/76	116/74	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	DSS	DSS	DSS	DSS
106/70	110/70	110/72	110/70				N	N	N	N				N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
112/72	118/70	120/70	116/72	114/70			N	N	N	N	N			N	N	N	N				N	N	N	N				Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
102/68	120/80	118/70	112/74	116/72	114/70		N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs
100/68	110/68	112/70	114/72	118/70			N	N	N	N	N	N		N	N	N	N	N	N		N	N	N	N	N	N		Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs
98/60	92/60	94/60	96/62	98/60	102/62	100/60	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	N	N	DSS	DSS	DSS	DSS
112/72	102/72	102/70	108/74	104/72			Y	Y	Y	N	N																				

Day5	Day6	Day7	Platlet Count(Thousands)							PCV(Packed Cell Volume)							Inotropic Support	RDP Transfusion	SDP Transfusion	Outcome
			Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day1	Day2	Day3	Day4	Day5	Day6	Day7				
Dengue fever without warning signs	Dengue fever without warning signs		26	18	29	67	121	184		46.2	39	42	42.3	38.3	38.1		N	N	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		29	12	33	46	134	181		42.9	37.1	36.3	37.1	35.2	32.9		N	N	Y	Discharged
Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs	24	29	39	69	108	140	182	48.5	46.5	45.5	43.6	44.9	41.2	39.5	N	N	N	Discharged
Dengue fever without warning signs			240	224	211	198	178			33.7	34.4	34.7	33.6	38.6			N	N	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs	Dengue fever without warning signs	155	137	120	104	162	185	194	34.6	33.6	35.5	36.2	35.8	33.2	32.1	N	N	N	Discharged
DSS	DSS	Dengue fever with warning signs	8	19	31	64	77	86	98	45.5	43.8	35.8	37.1	37.2	36.4	35.2	Y	Y	N	Discharged
Dengue fever without warning signs			76	69	94	135	215			42	43.9	41.5	37.7	38.9			N	N	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		26	19	51	46	75	127		46.7	44.3	41.9	39.2	37.9	42.8		N	Y	Y	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		8	12	22	38	101	224		42.8	37.8	34.7	31.8	33.6	31.9		N	Y	Y	Discharged
			32	34	61	102				40.9	36.4	38.2	36.8				N	Y	N	Discharged
			15							43.5							Y	Y	N	Expired
			38	45	83	187				31.3	31.9	32.4	33.5				N	N	N	Discharged
Dengue fever with warning signs	Dengue fever with warning signs		49	28	23	36	59	115		31	34.9	29.9	29.4	27.9	28.4		N	Y	N	Discharged
Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	16	13	20	46	127	181	271	43.8	42.7	41.5	39.6	35.3	37.2	37.6	N	Y	N	Discharged
DSS	DSS	DSS	12	48	98	177	189	223	254	37.7	37.3	45.2	42.1	40	38.3	37.4	Y	Y	Y	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		14	14	38	65	66	94		28.8	29.3	26.6	27.8	24.9	26.5		N	Y	Y	Discharged
Dengue fever with warning signs	Dengue fever with warning signs		9	22	34	48	179	212		47.3	30	38.3	38.9	39.1	38.6		N	Y	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		137	112	94	90	126	166		40.8	41.3	40.9	41.2	40.4	38		N	N	N	Discharged
DSS	DSS	DSS	11	52	46	52	51	58	66	35	38	34.5	30.4	28.6	30.2	26.4	N	N	N	Discharged
			26							44.8							Y	N	N	Expired
			18	52	128					41.7	44.4	36					Y	Y	N	Expired
			23	19						45.3	35.1						Y	Y	Y	Expired
Dengue fever without warning signs			20	54	45	63	104			33.6	31.3	27.7	26.4	26.8			N	Y	N	Discharged
			16	14	56	128				32.4	33.2	30.6	33				N	Y		Expired
			88	132	111	178				32.1	40.1	41.3	38.2				N	N	N	Discharged
Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	128	118	103	50	89	126	193	9.2	13	20.9	30.2	28.7	29.3	28.7	Y	N	N	Discharged
Dengue fever without warning signs			14	18	45	55	123			47.6	48.1	40.8	38	44.5			N	N	N	Discharged
			12	35	72	114				41	45	39.7	38.3				N	N	N	Discharged
Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	23	14	83	130	96	94	106	51.3	34.2	25.1	23.8	21.7	21.2	35.4	Y	N	Y	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		63	41	15	24	53	85		38.5	38.5	43.8	36.5	35.1	35.7		N	N	N	Discharged
Dengue fever without warning signs			14	17	42	71	153			46.6	40.1	40.9	41.4	39.9			N	N	Y	Discharged
Dengue fever with warning signs	Dengue fever without warning signs		54	17	13	23	73	204		39.7	37	39.9	38.9	36.8	35.4		Y	Y	N	Discharged
Dengue fever with warning signs	Dengue fever with warning signs	Dengue fever without warning signs	129	119	92	30	18	17	82	35.3	35	37.1	31.2	41.1	37.9	27.3	Y	Y	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		123	97	61	15	46	102		37.9	34.6	34	23.3	34	34.4		N	Y	N	Discharged
Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	101	92	77	31	27	55	92	37.5	36.4	37.7	42	42.9	38.7	38.6	N	N	N	Discharged
Dengue fever without warning signs			36	30	45	90	170			48.9	41.3	44.3	42.2	41.6			N	N	N	Discharged
			161	111	105	118				40.3	39.2	35.4	35.6				N	N	N	Discharged
			18	8						35.3	37.4						Y	Y	N	Expired
Dengue fever without warning signs			26	44	49	83	140			28.9	31.5	26.9	28.3	28.3			Y	N	N	Discharged
			78							44.1							Y	N	N	Expired
DSS	Dengue fever with warning signs	Dengue fever with warning signs	15	8	25	94	179	247	256	44.4	44.3	39.2	37.8	37.9	35.7	38.2	Y	Y	N	Discharged
			16	45	60	139				36.4	34.9	32.5	37.5				N	N	N	Discharged
Dengue fever without warning signs			61	53	66	122	189			35.7	32.3	33.5	33.6	34.4			N	N	N	Discharged
			109	84	75	83				39.4	38.4	37.7	39.1				N	N	N	Discharged
			21	44	67	145				35.2	37.2	35.8	35				N	Y	N	Discharged
			28	50	115	290				31.8	33.1	30.8	34.6				N	Y	N	Discharged
			125	134	165	182				28	20.6	26.3	32.2				N	N	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		105	67	52	50	70	83		47.2	45.1	41.5	40.2	39.4	37.4		N	N	N	Discharged
			136	95	68	80				42	42.1	41.8	40.2				N	N	N	Discharged
			152	152	128	111				42.5	44.9	44.5	46.2				N	N	N	Discharged
DSS	DSS	DSS	26	54	40	100	79	43	101	34.3	20.7	28.4	25.6	27.7	30.8	29.3	Y	Y	Y	Expired
			21	45	79	160				41.2	37	34.8	34.1				N	N	N	Discharged
Dengue fever without warning signs			13	10	14	51	67			40.2	40.6	39.8	39	35.7			N	N	N	Discharged
Dengue fever without warning signs	Dengue fever without warning signs		16	10	39	44	88	143		47.7	49	43.3	45.5	45.9	44.9		N	Y	Y	Discharged
Dengue fever without warning signs			30	25	70	74	104			49.9	50	43.6	42.7	43.6			N	N	Y	Discharged
Dengue fever with warning signs	Dengue fever without warning signs	Dengue fever without warning signs	170	140	96	64	41	73	170	34.2	33	32.6	33.3	33.1	36.3	35	Y	N	N	Discharged
Dengue fever without warning signs			26	30	68	81	158			40.8	40.6	37.5	37	31.9			Y	N	N	Discharged
			156	116	94	110				46	40.4	42.6	41.4				N	N	N	Discharged
Dengue fever without warning signs			18	25	20	42	121			44.6	32.6	35.1	32.8	35.5			Y	Y	N	Discharged
Dengue fever without warning signs			39	13	49	55	1110			43.8	26.6	45.9	38.8	37.8			Y	N	Y	Discharged