"UTILTY OF TOTAL LEUCOCYTE COUNT, HEMATOCRIT AND PLATELET INDICES IN PREDICTING THE SEVERITY OF DENGUE INFECTION''

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I hereby declare that this dissertation entitled **"UTILTY OF TOTAL LEUCOCYTE COUNT, HEMATOCRIT AND PLATELET INDICES IN PREDICTING THE SEVERITY OF DENGUE INFECTION"** is a bonafide and genuine research work carried out by me under the guidance of **DR. SUREKHA B.HIPPARGI** Professor, Department of Pathology B.L.D.E.U(Deemed to be University) Shri B.M.Patil Medical College, Hospital & Research centre, Vijayapura, Karnataka.

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Date: **Dr. Moksha S**

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LIST OF ABBREVATIONS USED

DEN 1	Dengue Virus Type 1
DEN 2	Dengue Virus Type 2
DEN 3	Dengue Virus Type 3
DEN 4	Dengue Virus Type 4
MPV	Mean Platelet Count
PDW	Platelet Distribution Width
P-LCR	Platelet large cell ratio
PCT	Plateletcrit
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DIC	Disseminated intravascular coagulation
DSS	Dengue shock syndrome
EIA	Enzyme immuno assay
ELISA	Enzyme linked immunosorbent assay
IgM/IgG	ImmunoglobulinM/ImmunoglobulinG
IL	Interleukin
TNF	Tumor Necrosis Factor
KD	Kilo Dalton
NVBDCP	National Vector Borne Disease Control Programme
PCR	Polymerized chain reaction
APA	Anti platelet antibody
MI	Mosquito inoculation

TC	Tissue culture
ISH	In Situ Hybridization
CFT	Complement fixation test

ABSTRACT

INTRODUCTION- Dengue infection is the most rapidly spreading mosquito borne viral disease in the world. In dengue infection clinical illness ranges from inapparent or mild febrile illness such as dengue fever (DF) to its complications such as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Early recognition of shock or hemorrhage and appropriate management with fluids prevents morbidity to a great extent. In this study we attempted to evolve a simple hematological markers for prediction of severe dengue.

OBJECTIVE- To know the utility of total leucocyte count, hematocrit and platelet indices in predicting the severity of dengue infection.

MATERIALS AND METHODS- Patients from both out-patient and in-patient departments, referred to the Department of Pathology in BLDE (Deemed to be university) Shri B.M.Patil Medical College, Hospital and Research centre, Vijayapura. All the blood samples of cases who were serologically positive for dengue infection were collected in k2 EDTA vacutainers and are processed by Sysmex XN1000 haematology analyser which is based on the principle of fluorescence flow cytometry.

RESULTS –The study population consisted of 114 dengue fever cases and 9 cases of dengue hemorrhagic fever. Thrombocytopenia was found in 89.47% cases of DF and 100% cases of DHF. A low PCT was found in 42.1% cases of DF and 66.67% cases of DHF. High PDW was observed in 36.84% of DF and 88.89% DHF cases. Raised hematocrit was found in 35.08% cases of DF and 55.55% cases of DHF. Leucopenia was observed in 52.6% cases of DF and 55.55% cases of DHF.

CONCLUSION- In the present study it is emphasized that basic hematological parameters can be used as indicators of dengue infections. Since DF does not have a specific medical therapy, clinical recovery monitoring is largely dependent on hematological parameters. Thus, these parameters can be used as screening tools by physicians to chart early therapeutic response.

KEY WORDS- DF, DHF, DSS, Hematological parameters.

TABLE OF CONTENTS

Sl. No.	Contents	Page No
1.	INTRODUCTION	1
2.	OBJECTIVE	3
3.	REVIEW OF LITERATURE	4
4.	MATERIALS AND METHODS	29
5.	RESULTS	31
6.	DISCUSSION	45
7.	SUMMARY	54
8.	CONCLUSION	55
9.	REFERENCES	56
10.	ANNEXURES	64

LIST OF TABLES

Sl. No	Title of Tables	Page No.
1	Yearly prevalence of various dengue serotypes in India	6
2	Cytokine profile in patients with dengue infection	10
3	Distribution of cases according to gender	31
4	Distribution of cases according age	32
5	Distribution of cases according to platelet count	33
6	Distribution of cases according to MPV	35
7	Distribution of cases according to PDW	37
8	Distribution of cases according to PLCR	38
9	Distribution of cases according to PCT	39
10	Distribution of cases according to Hematocrit	40
11	Distribution of cases according to Total leucocyte count	41
12	Distribution of cases according to Dengue serology positivity	42
13	Comparison between dengue fever and dengue hemorrhagic fever	44
14	Comparision of Gender wise distribution with other studies	45
15	Comparision of Clinical Spectrum with various studies	46
16	Comparision of Thrombocytopenia in DF with other studies	47
17	Comparision of Thrombocytopenia in DHF with other studies	48
18	Comparision of MPV,PDW in DF with other studies	49
19	Comparision of MPV,PDW in DHF with other studies	49

20	Comparision of Plateletcrit in Dengue Infection with other studies	50
21	Comparision of P-LCR in Dengue Infection with other studies	51
22	Comparision of Hematocrit in DF with other studies	51
23	Comparision of Hematocrit in DHF with other studies	52
24	Comparision of Leucopenia in DF with other studies	52
25	Comparision of Leucopenia in DHF with other studies	53

LIST OF FIGURES50

Sl. No	Title of Figures	Page No.
1	Bar Graph showing dengue incidence rates (per million population) in India from 1998 to 2014	6
2	Schematic representation of dengue genome	7
3	Dengue envelope and virion structure	8
4	Transmission cycle of dengue virus involving mosquitoes and susceptible hosts	9
5	Mechanism of action of dengue virus	11
6	Mechanism of action of dengue virus	11
7	Euro Immune ELISA with kit	26
8	Bar Graph Showing Distribution of cases according to gender	31
9	Bar Graph Showing Distribution of cases according age	32
10	Bar Graph Showing Distribution of cases according to platelet count	34
11	Bar Graph Showing Distribution of cases according to MPV	36
12	Bar Graph Showing Distribution of cases according to PDW	37
13	Bar Graph Showing Distribution of cases according to PLCR	38
14	Bar Graph Showing Distribution of cases according to PCT	39
15	Bar Graph Showing Distribution of cases according to Hematocrit	40
16	Bar Graph Showing Distribution of cases according to Total leucocyte count	41
17	Bar graph showing distribution of patients according to dengue serology in DF and DHF	43

INTRODUCTION

In India dengue viral infection is known to exist for a long time.[1] Dengue fever is the most common arboviral disease in the world, and presents cyclically in tropical and subtropical regions of the world. The four serotypes of dengue virus are 1,2,3 and 4 and they form an antigenic subgroup of the flavi viruses (Group B arboviruses).[2]

Transmission to humans of any of these serotypes initiates a spectrum of host responses, from inapparent to unpredictable clinical evolution and outcome. The geographic distribution of dengue virus has greatly expanded and the number of cases of dengue fever (DF) and its severe manifestations like dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) has dramatically increased during the past three decades.[2]

The initial symptoms of dengue are often nonspecific. Viremia may be below detectable levels and serological tests confirm dengue late in the course of illness making early recognition of dengue challenging.[3]

Thrombocytopenia, raised hematocrit, lymphocytosis especially atypical lymphocytosis and neutropenia are the consistent findings in dengue.[4]

Few studies have shown significant change in platelet indices (MPV, PDW, PCT, P-LCR) and these changes have been correlated with severity of disease hence been considered as prospective platelet activation markers in dengue infection.[3,5]

MPV can be used as an independent predictor of bleeding. When MPV increases it indicates increased megakaryocytic activity and when MPV decreases it indicates marrow suppression and increased risk of bleeding. Platelet activation causes

morphologic changes of platelets including both spherical change and pseudopodia formation. Platelets with increase in number and size of pseudopodia differ in the overall size of the platelet, affecting PDW. Plateletcrit(PCT) is used as a reliable measure of platelet biomass as it combines both MPV and absolute platelet count. Platelet large cell ratio(P-LCR)was significantly decreased in patients with thrombocytosis when compared to normal while it was increased in thrombocytopenia showing an inverse relation to platelet count. But is directly related to MPV and PDW.[3,5]

Since specific medical therapy is not available for dengue fever, clinical recovery monitoring is largely dependent on hematological parameters.[6]

Thus the need of the study is to evaluate the platelet indices, hematocrit and total leucocyte count in patients with dengue infection and to know the role of these indices in assessing severity of dengue viral infection which will be of great help in limiting morbidity and mortality associated with dengue infection.

OBJECTIVE OF THE STUDY

To know the utility of total leucocyte count, hematocrit and platelet indices in predicting the severity of dengue infection.

REVIEW OF LITRATURE

HISTORY

The word dengue means a sudden cramp like seizure which is believed to be originated from the Swahili phrase “Ki Denga Pepo”. Dengue fever was first referred to as a “water poison” which was mentioned in a Chinese medical encyclopedia in 992(265-420 AD) and was thought to be associated with flying insects.[7]

The first clinically recognized Dengue epidemics occurred in the 1780s involving the three countries Asia, North America, Africa almost simultaneously.

It was surmised that DENV was the etiological agent during the disease outbreaks that occurred in French Westindies in1635, in Panama in 1699, Philadelphia epidemic in 1700s.

The first clinical case was reported the year 1789 by Benjamin Rush. He also named the term “Break Bone Fever” due to symptoms such as myalgia and arthralgia. It was after 1828 the term dengue fever came into routine use.[8]

Thus before the 18th century Dengue was believed to have a very wide geographical distribution.[8]

In 1906 Thomas Lane Bancroft put farward a consideration that Ades Ageypti was involved in the transmission of dengue infection.[9]

In 1939-1945 during the world war II, the ecological disruption which occurred made the path smooth for increased transmission of mosquito borne diseases. As a result

there was increased incidence of complicated dengue cases and multiple strains of dengue virus also have become endemic in South East Asia and pacific.[10]

During 1953-54 the first major epidemic of DHF took place in phillipines and thus followed by a quick global spread of epidemics of DF/DHF.

In the year 2000 and from past 50 years it was stated that the incidence of dengue was dramatically increased and has become endemic in more than 100 countries.[11]

In 2017 the trends indicate that in the past decade, the escalation of dengue as a threat to health, finance, and health services has increased substantially. This mosquito-borne viral infection has grown 30-fold since it was first reported and then expanded and diversified globally. There is an increasing new trend of concurrent multiple DENV, CHIKV, and ZIKV in hyperendemic dengue infection areas. [12]

Epidemiology of dengue in India

In 1780 the first clinical dengue like illness was recorded in Madras (now Chennai). The first virologically proven dengue was recorded in Calcutta and Eastern coast of India in 1963-64[13]. It extended towards northward and spread to Delhi in 1967 and Kanpur in 1968. At the same time it also spread to southern part of India and also gradually involved the whole country with wide spread epidemics.[14] It was followed by endemic/hyperendemic occurrence of all the four serotypes of DV.

In 1968 the epidemic that occurred in Kanpur was due to DV-4[15]. During 1988-89 when the epidemics occurred in rural and urban areas of Gujarat, DV-2 was isolated. Outbreaks of DF which occurred in Rajasthan were by DV-1 and DV-3, outbreaks that occurred in Madhya Pradesh were by DV-3[16] . DV-1 was isolated during epidemics occurred in Delhi in 1997.[17]

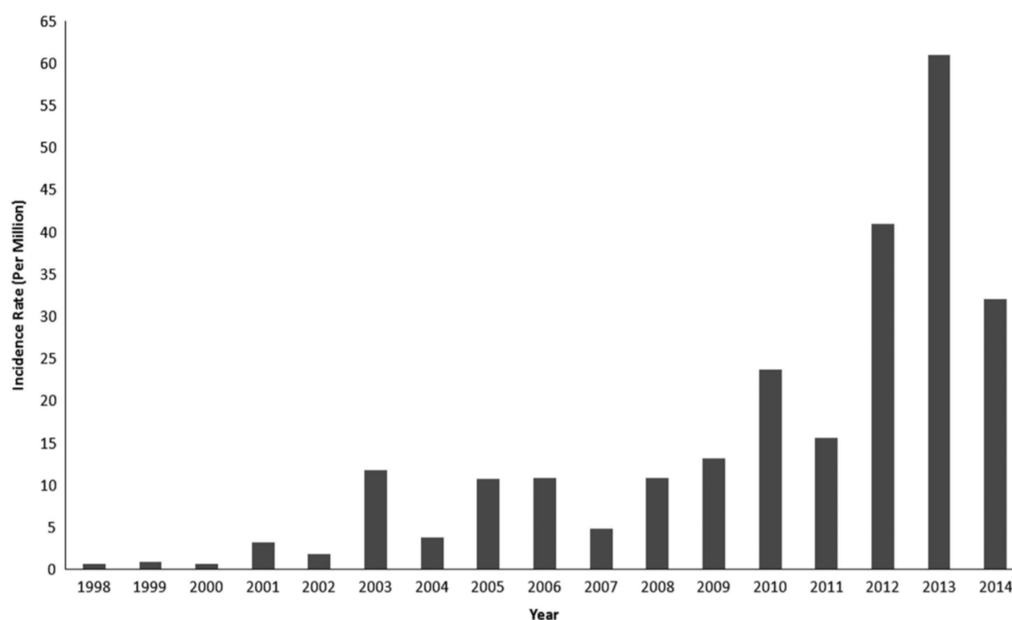


FIGURE 1: DENGUE INCIDENCE RATES (PER MILLION POPULATION) IN INDIA FROM 1998 TO 2014[18]

The incidence of Dengue infection raised sharply from 1998 to 2001 from 0.72 to 3.21 per million population. But since 2010 the incidence of dengue exceeded to a greater extent of about 15 per million population which was recorded annually.[18]

Table1: Yearly prevalence of various dengue serotypes in India[1]

Year	State	Prevalent serotype
1964	Tamil Nadu	2
1968	Tamil Nadu	1, 2, 3, and 4
1970	Uttar Pradesh	1, 2, 3, and 4
1996	Uttar Pradesh	2
1996	Delhi	2
1996	Haryana	3
1997	Delhi	1
2001	Madhya Pradesh	2
2003–2005	Delhi	1, 2, 3, and 4
2007–2009	Delhi	1, 2, 3, and 4
2009–2010	Maharashtra	4
2010–2011	Delhi	1
2009–2012	Uttar Pradesh	1, 2, and 3

DENGUE VIRAL STRUCTURE

Dengue virus is a ss RNA virus and belongs to the Flaviviridae family and Flavivirus genus.[20]

The Genome encodes polyprotein precursor of approximately 3,400 amino acid residues and is about 11kb long. Three structural proteins are generated by Co and post translational processing by cellular and viral proteases. They are C(Capsid) protein, M(Membrane) protein, S(Structural)protein and seven non structural proteins i.e NS1,NS2a,NS2b,NS3,NS4a,NS4b,NS5.[21]

The viral particle contains the RNA genome and it forms the inner core surrounded by C proteins[22]

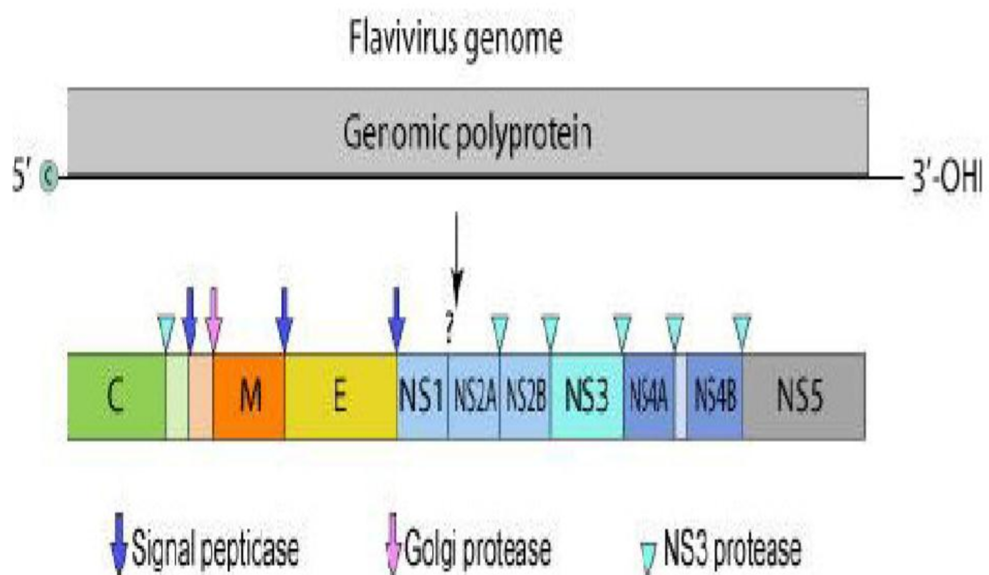


Figure 2: Schematic Representation of Dengue Genome

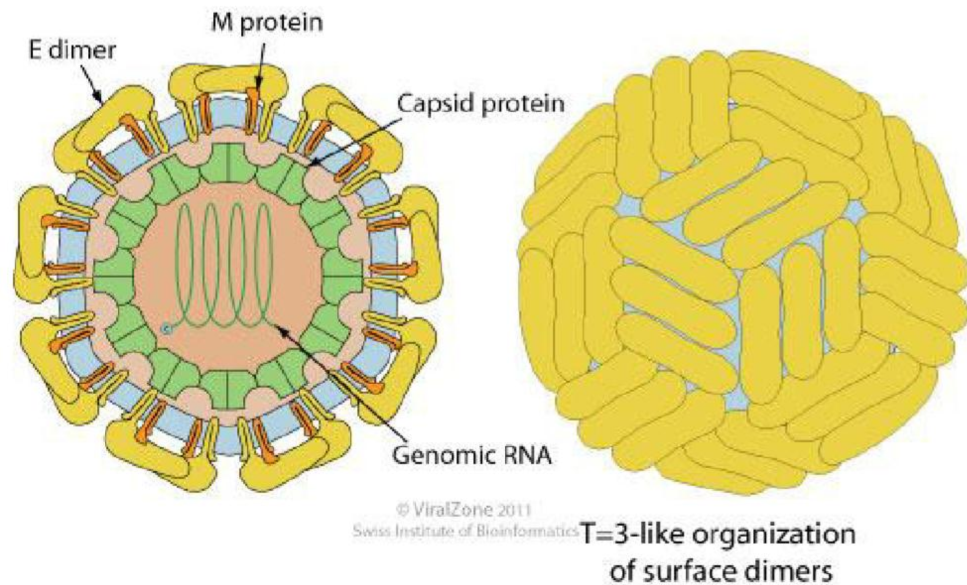


Figure 3: Dengue Envelope and Virion Structure[22]

Vectors of Dengue Virus:

Dengue virus is most commonly transmitted by Aedes aegptyi and rarely by other species named Ades albopictus.

Aedes aegptyi has both tropical and sub tropical distribution and it's a small black and white colored mosquito. The ideal larval habitants for this vector are both natural and artificial reservoirs for water(water storage containers, flower pots, discarded tires, buckets, tin cans, drums, water baths for pets) which are nearby to places where humans live.[23]

The mosquito bites most commonly during day time and its primarily active for appropriately two hours following sunrise and several hours after sunset.[23]

Transmission occurs from one infected human to a mosquito, which later becomes infected and transmits the infection to second human[24]

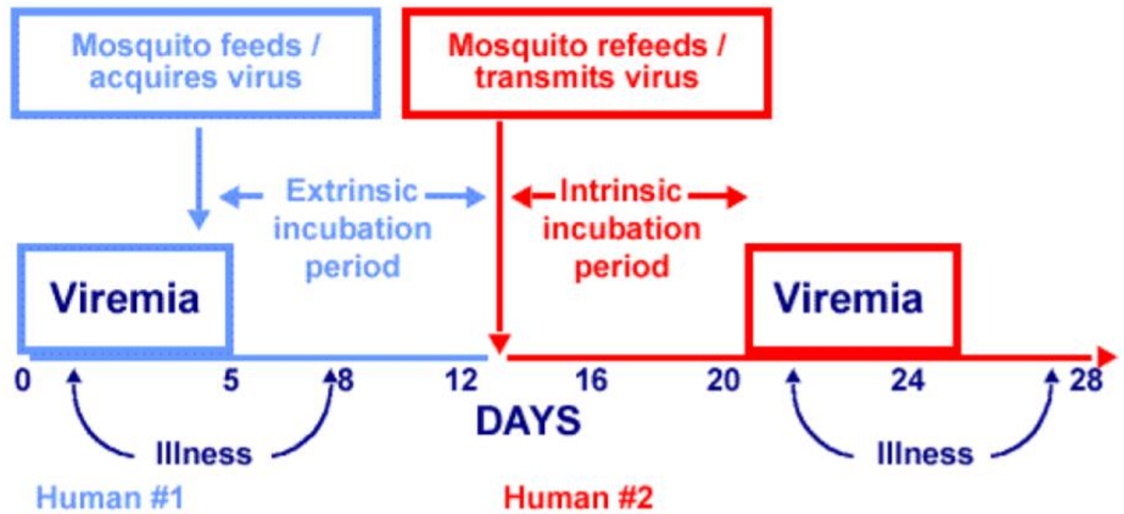


Figure 4: Transmission cycle of DENV involving mosquitoes and susceptible hosts[24]

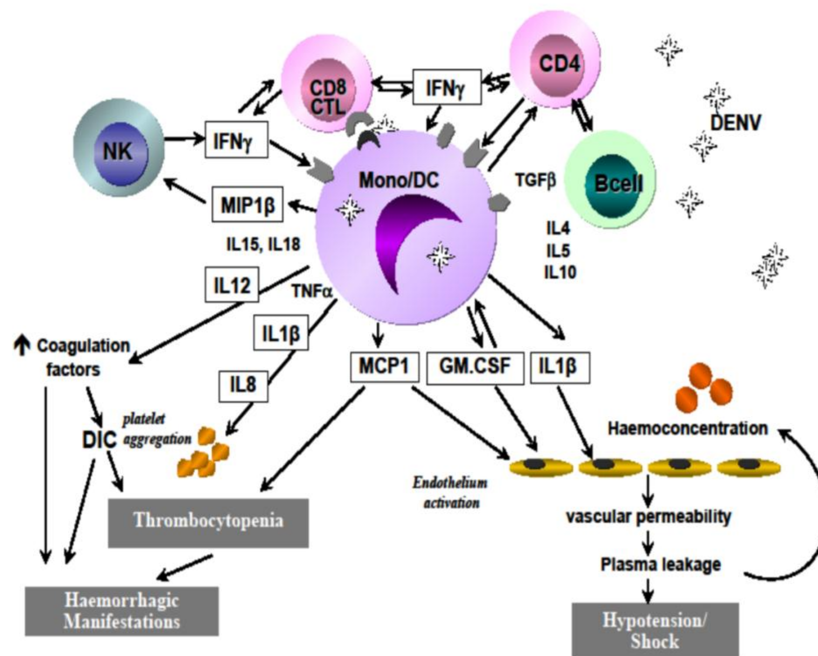
PATHOGENESIS

When an Aedes aegytpti feeds on the human, it injects the virus into the blood stream. The virus targets the immature langerhans cells and keratinocytes. The Infected cells then migrate to lymph nodes, and the virus then attacks monocytes and macrophages. Consequently, the infection is replicated and virus migrates to various parts through the lymphatic system. The presence of the viruses in the blood stream is known as viremia. As a result of this viremia, many other cells get infected including blood-derived monocytes, myeloid dendritic cells, and splenic and liver macrophages. Macrophages and lymphocytes are mainly infected with the virus. Viremia occurs within two to six days of infection. Studies have reported high level of viremia in DHF patients as compared to DF patients.[25]

Dengue virus replicates in the macrophage like cells and induce quickly the CD4+ T cells to produce unique cytokine factor, the cytotoxic factor (hcF) which in turn induces the macrophage to produce free radicals, reactive oxygen, and peroxynitrate. The free radicals then kills the target cells by apoptosis and they also directly up regulate the production of pro inflammatory cytokines IL-1, TNF-α, IL-8 and H2O2 in macrophages.

TABLE 2: CYTOKINE PROFILE IN PATIENTS WITH DENGUE INFECTION[26]

Cytokines	Dengue fever	DHF
IL-1	markedly↑	↑
IL-2	↑	Markedly ↑
IL-4	↑	Markedly ↑
IL-8	↓	Markedly ↑
IL-10	↓	Markedly ↑
IL-12	Markedly ↑	↓
IL-13	↑	Markedly ↑
IL-18	↑	Markedly ↑
INTERFERON- α	Markedly ↑	↑
TNF- α	Markedly ↑	Markedly ↑
TRANSFORMING GROWTH FACOR	↓	Markedly ↑
CYTOTOXIC FACTOR	↑	Markedly ↑



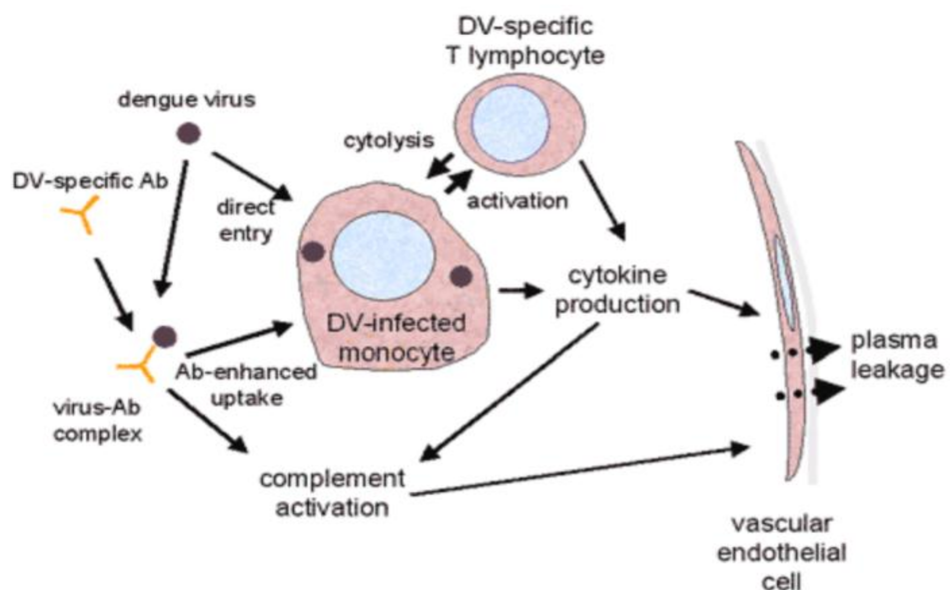


Figure 5(above) Figure 6(below) Showing mechanism of action of dengue virus.[26]

The elevated concentrations of circulating pro inflammatory mediators that result from viral replication with in the macrophages, an immune response to this, or a more direct viral damage to the endothelial cells leads to increased vascular permeability observed in patients with DHF.

A rapid massive but self limiting further increase in micro vascular permeability may be superimposed on this background of increased permeability. This would allow plasma water to go out of intra vascular compartment.[27] During acute phase of DHF circulating levels of complement anaphylatoxins, cytokines, chemokines were found to be increased. The peak increase in cytokines particularly C3A and C5A coincide with the occurrence of shock leakage which are the manifestations leading to death or morbidity.

Activation was believed to be triggered by many mechanisms including antigen-antibody complex. Antibody binding to dengue infected cells subsequently leads to complement activation. Endothelial cells and other target cells are also activated by dengue virus infection. Dengue NS-1 is a 45 KD non structural protein which resides in the plasma membrane of infected cells.

High levels of NS-1 are found in the circulation of dengue virus infected patients during acute phase of disease. NS-1 activates human complement and thus large amounts of complement activation probably occurs at the site of vascular leakage.Complement anaphylatoxins increase vascular permeability. [26]

Drowsiness is found to be most commonly associated with patients who develop shock. This could be due to cerebral hypo perfusion.[28]

Thrombocytopenia along with an imbalance in the regulation of coagulation and fibrinolysis as in disseminated intravascular coagulation syndrome (DIC) is considered to be the cause for bleeding tendency in DHF. [29]

The circulating antibodies has two biological activities; neutralization of virus and amplification of infections. In Thailand fourteen DHF/DSS occured in infants during DEN-2 virus infection, when maternal and neutralizing antibodies has metabolised to low titre and infections enhancing antibodies were left in circulation.[30]

CLINICAL FEATURES

Dengue viral infection may be asymptomatic or may cause undifferentiated febrile illness (viral syndrome), dengue fever(DF), dengue hemorrhagic fever(DHF), including Dengue Shock syndrome(DSS).The clinical manifestations depends on virus strains and host factors such as age, immune status.[31]

Infection with one dengue serotype gives lifelong immunity to that particular serotype, but the cross protection for other serotypes is only for short duration.[32]

"Manifestations of Dengue Virus

1) Asymptomatic
2) Symptomatic

A)Undifferentiated

B)(Classical) dengue fever

C)Dengue Haemorrhagic Fever(DHF)

- No shock
- Dengue Shock Syndrome(DSS)

Asymptomatic Infection

Almost around one half of individuals infected with dengue are asymptomatic, that is they show no clinical signs and symptoms.

Undifferentiated Fever

The first clinical course is a relatively benign scenario where the patient experiences fever with mild non specific symptoms that may mimic any number of other acute febrile illnesses. They do not meet case definition criteria for DF.

Dengue Fever

Dengue fever consist of

(1) **Febrile phase :** During first 2-7 days Manifestations

- Temperature-39-40 c
- Head ache
- Retro orbital pain
- Muscle pain Joint/Bone pain
- Flushed pain
- Rash
- Skin hemorrhage
- Bleeding

(2) Afebrile phase (critical phase): During 2-3 days after febrile phase manifestations:

- Same as during febrile phase
- Improvement in general conditions
- Appetite rapidly regenerated.

(3) Convalescence phase : During 7-10 days after critical stage

- Further improvement in general conditions
- Return of appetite
- Bradycardia
- Confluent petechiael rash"[33]

The characteristic features of Classical dengue fever include acute onset of fever, and may be associated with chills. This fever persists for 5-7 days, later it undergoes remission for few hours to few days, after which fever may appear again and therefore it is named 'saddle back fever'. Fever is accompanied by the presence of severe frontal headache, retro orbital pain, severe musculoskel*et al* pain, lumbar back pain, pain abdomen(break bone fever).Anorexia, nausea, vomiting and hyperesthesia may also be present.Initially skin appears flushed but in 3-4 days after the fever starts declining maculopapular rash appears. It spares the palms and soles. Even though the rash fades, petechiae may persist on the limbs. After these episodes fever reappears which is later followed by recovery.[34]

In addition to the above features **Dengue Hemorrhagic Fever** also exhibits the appearance of hemorrhagic manifestations, while dengue shock syndrome shows manifestations of shock, capillary leakage and altered mental status.[35]

DHF is caused by more than one dengue virus serotypes and is a severe form of DF. The infection initially will sensitize the patient and later it appears to produce an immunological event causing great and usually sudden damage or suffering. Illness starts with sudden fever, headache and facial flushing. It is also accompanied with anorexia, vomiting, epigastric discomfort, right costal margin tenderness and general

abdominal pain. DHF is characterized by plasma leakage, abnormal hematocrit manifested by raising hematocrit and moderate to marked thrombocytopenia.[25]

DENGUE SHOCK SYNDROME

Initial stages of DSS appears similar to DF/DHF. After 2-7 days when the fever starts declining, there is decrease in the perfusion leading to early signs of shock characterized by central cyanosis, diaphoresis, restlessness and cool, clammy skin and extremities. Gradually the pulse becomes weak and rapid, there is also narrowing of pulse pressure to less than 20 mmhg in the extremities later the blood pressure becomes unobtainable. Petechiae as well spontaneous ecchymosis occurs, with decrease in platelet counts along the mucosal sites like for example gastrointestinal bleeding.75% of the cases show hepatomegaly with variable splenomegaly.

80% of the cases show pleural effusion. With capillary alveolar leak adult respiratory distress syndrome may manifest. When left untreated there occurs hypoperfusion of heart and organ failure, in such cases fatality rate is 50%. On the other hand with proper treatment fatality is reduced to 1%.[33]

Usually dengue fever manifests as described above. Rarely it may present unusually with atypical symptoms like:[36,37,38]

- Encephalopathy- occurs due to hyponatremia, cerebral edema, microvascular frank hemorrhage. 0.5-6.2% of patients with DHF develop this form of dengue virus infection. Mortality rate is 22%.
- Cardiomyopathy/myocarditis[39]
- Mononeuropathy, polyneuropathy, Gullian barrie syndrome, transverse myelitis
- Rhabdomyolysis

- Liver failure
- Acute renal failure

DENGUE FEVER :CLINICAL DISCRIPTION

An acute febrile illness of 2-7days duration with two or more of the following manifestations: Headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations.

"CRITERIA FOR DENGUE HAEMORRHAGIC FEVER (DHF):

a) A case with clinical criteria of dengue fever

Plus

b) Haemorrhagic tendencies evidenced by one or more of the following

1.Positive tourniquet test

2.Petechiae, ecchymosis or purpura

3.Bleeding from mucosa, gastrointestinal tract, injection sites or other sites

Plus

c) Thrombocytopenia(<100000cells/cumm)

Plus

d) Evidence of plasma leakage due to increased vascular permeability, manifested by one or

more of the following:

1.A rise in average hematocrit for age and sex _>20%

2.A more than 20% drop in hematocrit following volume replacement treatment compared to baseline

3.Signs of plasma leakage (pleural effusion, ascites, hypoproteinemia)

CRITERIA FOR DENGUE SHOCK SYNDROME(DSS):

All the above criteria for DHF with evidence of circulatory failure manifested by rapid and weak pulse and narrow pulse pressure or hypotension for age, cold and clammy skin and restlessness."[33]

The new WHO classification for dengue severity is divided into Dengue without warning signs, Dengue with warning signs and severe dengue.

"Dengue without warning signs

Fever and any two of the following:

Nausea, vomiting

Rash

Aches and pain

Leucopenia

Positive torniquite test.

Dengue with warning signs

Dengue as defined above plus any of the following

Abdominal pain or tenderness

Persistent vomiting

Clinical fluid accumulation (ascites, pleural effusion)

Mucosal bleeding,

Lethargy, restlessness

Liver enlargement >2cm.

Laboratory: Increase in hematocrit with concurrent decrease in platelet

Severe dengue expanded

Dengue with atleast one of the following criteria

- Severe plasma Leakage leading to
- Shock
- Fluid accumulation leading to respiratory distress
- Severe bleeding as evaluated by clinician
- Severe organ involvement
- CNS impaired consciousness
- Failure of Heart and other organs”[31,33]

LABORATORY DIAGNOSIS OF DENGUE

HEMATOLOGICAL PARAMETERS like total WBC count, differential WBC count and platelet count are altered in dengue fever. The most common findings are[40].

1. Thrombocytopenia
2. Leucopenia
3. Lymphocytosis with reactive lymphocytes
4. Increased hematocrit (due to hemoconcentration)

1) PLATELET INDICES

A) THROMBOCYTOPENIA-

The most common laboratory abnormality in dengue is thrombocytopenia and the mechanism for thrombocytopenia is multifactorial.

1. Because of direct damage to the megakaryocyte production there is decreased platelet production.

2. The pre-existing antibodies leads to the formation of immune complexes with the viral antigen and leads to increased peripheral destruction of platelets.

3. NS1 antigen also produces antiplatelet antibodies(APA) which cross reacts with integrins and adhesins and leads to platelet aggregation.

4. Complement mediated lysis also has an important role in thrombocytopenia.[40]

A platelet count of 1.5-4lakh/cumm was considered normal

Vulavala S *et al* conducted a study on clinical and laboratory profile of dengue fever patients and found thrombocytopenia in 78.06% of cases. They also stated bleeding diathesis in dengue fever could be due to low platelet count and leakage from vessels.[41]

B) MPV- MPV is determined in the progenitor cell, the bone marrow megakaryocyte. When platelet production is decreased young platelets become bigger and more active, and MPV levels increase. Increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation platelets shape change from biconcave discs to spherical, and a pronounced pseudopod formation occurs that leads to increase in MPV during platelet activation.[42]

Mean platelet volume, normally measured using automated blood analysers, reflects the average size of platelets in circulation. It is meant to show the relationship between platelet synthesis in bone marrow and cell destruction. A normal MPV has a range of 7.5-11.5fl. MPV correlates with platelet function and may be more sensitive than platelet count as a biomarker in variety of diseases. It is also regarded as a useful surrogate marker of platelet activation or reactivity.

The newer machines are offering an ever increasing range of modalities for hemogram and platelet analysis. They use different principles such as electrical impedence, optical light scatter, and fluroscent staining for studying blood cells which are giving encouraging results.[42,43]

A study done by Bashir AB *et al*[5] on Role of Platelet Indices in Patients with Dengue Infection in Red Sea State found that MPV was decreased in cases of study group that is dengue positive cases and was normal in cases of control.

C) PLATELET DISTRIBUTION WIDTH (PDW)- It is an indicator of volume variability in platelet size and is increased in the presence of platelet anisocytosis. PDW is a distribution curve of platelets measured at the level of 20% relative height in a platelet size distribution curve, with a total curve height of 100%. The PDW reported varies markedly, with reference intervals ranging from 10-14fl. PDW directly measures variability in platelet size, changes with platelet activation and reflects the heterogeneity in platelet morphology.

A study done by Bashir AB *et al*[5] on Role of Platelet Indices in Patients with Dengue Infection in Red Sea State found that PDW was increased in cases of study group that is dengue positive cases and was normal in cases of control.[42]

D) PLATELET LARGE CELL RATIO(P-LCR)- It is an indicator of circulating large platelets(>12 fl) which is presented as percentage. The Normal percentage range is 11-33%. It has also been to monitor platelet activity.[42]

E) PLATELETCRIT- Plateletcrit is the volume occupied by the platelets in the blood as a percentage and calculated according to the formula PCT=platelet count×MPV/10,000. Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination.[42]

2) LEUCOPENIA Leucopenia, defined as decrease in total leucocyte count is a prominent and supposedly the second most common feature in dengue. Leucopenia is caused by bone marrow suppression by virus in acute phase and is due to decrease in polymorphs.

Neutropenia is also attributed to marked degeneration of mature neutrophils in febrile phase with shift to left. Stress accompanied with shock may be the cause of mild initial leukocytosis.[44]

Kailash CM *et al* in their study stated that the leucoyte count ranged from 1310-16700 cells/cmm with a mean leucocyte count of 4701.2cell/cmm. They found that leucopenia was found to be present in among 51% of cases.[45]

3) LYMPHOCYTOSIS :

Atypical lymphocytes Atypical lymphocytes are usually seen in a number of viral infections like infectious mononucleosis, viral hepatitis herpes, influenza and rubella. “The exact function of atypical lymphocytes is unclear, they incorporate increased amounts of [3H] thymidine into deoxyribonucleic acid and are similar in appearance to lymphocytes which undergo blast transformation after stimulation with mitogens (such as phytohemagglutinin).”[2] It is thus possible that atypical lymphocytes are regarded as a response to non-specific viral stimulation or to specific viral antigens due to recognition accompanied by transformation.[2]

Reactive lymphocytes plays a pivotal role in the immune response. The presence of atypical lymphocytes and their number provides useful details in certain disease specific states. Atypical or reactive lymphocytes are usually larger than a mature lymphocyte. The cell may be intended at its periphery by the surrounding cells giving it a scalloped appearance. The cytoplasm is abundant, often vacuolated and appears foamy. The nucleus is distinctive, it appears lobulated or indented. It may be oval, kidney shaped, round or placed eccentrically. The chromatin appears slightly fine when compared to that of a small lymphocyte.[46]

A number of studies have shown that early in the course of illness, patients with either primary or secondary dengue infections exhibit a fall in the leukocyte count associated with a rise in the percentage of lymphocytes and this finding is in parallel to marrow suppression during acute phase.[2]

4) HEMOCONCENTRATION- Hemoconcentration is a very important parameter. The raised hematocrit is an accurate indicator of vascular permeability and plasma leakage.[47]

Various pro inflammatory cytokines and inflammatory mediators like IL-8,C3a,C5a are responsible for capillary and plasma leak there by responsible for hemoconcentration.

Few studies have reported that fluid leakage does not achieve a high degree of hemoconcentration inspite of the patient being in shock because of associated anemia and blood loss.[47]

A hematocrit value of 36-46% was considered normal.

II) MICROBIOLOGICAL DIAGNOSIS

1. “Virus Isolation
2. Demonstration of viral antigen or RNA in tissue or serum
3. Demonstration of IgM antibodies or raising titre of IgG antibodies in paired sera against dengue virus i.e serological diagnosis.
4. Molecular diagnosis PCR, Serotyping, Genotyping”

1) **Virus isolation** - can be done by Mosquito inoculation(MI), Tissue culture(TC), Animal Inoculation techniques. Isolation and identification of the viruses is considered as gold standard and gives a conformatory diagnosis.[48]

2) Demonstration of viral antigen or RNA in tissue or serum

Viral antigen can be detected in various tissues like liver, spleen, lymphnode, thymus, kidney,lung, phagocytic cells, mouse brain squash by Immunofluoresence and IHC techniques.[49]

Other method of demonstrating viral antigens is by using monoclonal antibody in antigen capture Enzyme Linked Immunosorbent Assay (ELISA). It is used as an inexpensive way to screen large number of mosquito specimens, as well as human specimens. ELISA based methods which use specific monoclonal antibodies lead to a definite diagnosis.[49]

Viral RNA can be demonstrated in various infected tissues by In Situ Hybridization (ISH) technique. Intense positive staining suggest that viral replication may have occurred in these tissues.[49]

3) Serological Diagnosis:

Serological diagnosis depends on presence of IgM antibody or a rise in IgG antibody titre in paired sera.

"Detection of IgM antibodies:

IgM antibodies against dengue virus appear around five days after the onset of symptoms and are detectable for 1-3 months after acute episodes.

IgM antibodies can be detected by;

1) IgM capture ELISA test.
2) Rapid IgM strip test.
3) Dot ELISA.
4) Hemagglutination inhibition test – Seroconversion, or high titres (≥ 1: 2560)62is suggestive of acute infection.
5) CFT- Complement fixation tests
6) NT- Neutralization tests

Detection of IgG

IgG antibodies appear later than IgM antibodies in primary dengue infection;

Around six weeks later and persist at high levels for 30-40 days before levels decline. These antibodies persist for life. Detection of four fold or greater increase of IgG titre in paired serum sample, taken at an interval of 10-14 days confirms the diagnosis of dengue.

However in secondary dengue infection there is rapid rise of IgG ie, within 1-2 days and persists for 30-40 days.

IgG antibodies can be detected by;

1) IgG ELISA
2) Hemagglutination inhibition test (HI).
3) Dot ELISA.
4) Rapid IgG strip test.
5) Complement fixation test.
6) Neutralization test."[33]

Figure 7:Euro immune ELISA with kit

In secondary dengue infection IgG titre increases within 1-2 days of symptom onset and their levels persists for 30-40 days. IgM response is late or may not be detected at all.[50]Heterologous reactions with other Flaviviruses are problematic where numerous Flaviruses co-circulate, but in tropical Asia, only Japanese encephalitis and dengue viruses infect humans, the infections are easily distinguished.

4) Molecular diagnosis:

The viral genome can be detected by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). RT-PCR has provided one of the most important steps in the molecular detection of dengue virus.[51] First, the viral RNA is extracted from the specimen, then the complementary DNA (cDNA) is synthesized using reverse transcriptase enzyme, then the cDNA is amplified. The primary PCR product obtained from RT-PCR is further typed by nested PCR.

Jameel T *et al* [4] conducted a study on changing hematological parameters in dengue viral infection in the year 2010 and concluded that peripheral blood parameters like raised hematocrit, decrease in leucocyte count, presence of atypical lymphocytes, thrombocytopenia were very helpful in disease monitoring and can be useful in prediction of prognosis.

Bashir AB *et al* [5] in 2013 conducted a study on role of platelet indices in patients with dengue infection in red sea state, Sudan in 334 patients as clinical sample and 101 apparently healthy normal individuals as control sample and found out that MPV and platelet count were lower in dengue fever patients when compared to controls.

Shams N *et al*[52] conducted a study conducted a study on predictors of severity of Dengue Fever in Tertiary Care Hospitals, and among the 105 patients studied 75% were classified as DF, 23% as DHF, 2% as DSS. They had concluded that leucopenia, thrombocytopenia with platelet count less than 50,000 and presence of both dengue IgM and IgG can be used as predictor of progression to severe dengue.

Rathod PN *et al*[34] studied 100 patients and classified them according to the severity and concluded that laboratory parameters like presence of hemoconcentration,

decreased platelet count abnormal leucocyte count played an important role as predictors of dengue severity.

Navya B N *et al*[53] studied the role of platelet parameters in dengue Positive Cases which was an observational study in cases. They found out that significant difference was observed between severity of the thrombocytopenia and severity of the disease(p<-0.013).Among the 100 patients studied low MPV(<9fl) was seen in 72% of cases and high PDW (>13fl) was seen in 92% of the cases and concluded that low MPV and high PDW shows sensitivity for dengue fever thus reflecting a predictor marker for DF.

Lokanta H *et al*[47] evaluated the Laboratory Profile in Serologically proven dengue in children. They studied 100 children who were serologically proven for dengue and classified them as DF,DHF and DSS and concluded that presence of thrombocytopenia, hemoconcentration and leucopenia with reactive lymphocytosis were important findings in diagnosis of dengue.

Gajera VV *et al* [6] conducted a study on Study of Hematological profile of Dengue Fever and its Clinical Implication on 100 patients and concluded that thrombocytopenia was most predominant hematological finding. Raised hematocrit was not significant in their study and also found out that leucocyte count is an important benchmark for clinical improvement in case of dengue.

MATERIAL & METHODS:

SOURCE OF DATA:

Patients from both out-patient and in-patient departments, referred to the Department of Pathology in BLDE (Deemed to be University) Shri B.M.Patil Medical College, Hospital and Research centre, Vijayapura who were serologically positive for dengue were included in the study.

Study Period – 1st October 2016 to 31st August 2018.

- **Normal values**

  Platelet count-1.5-4lakhs/cmm

  MPV- 7.4-11.4 fl

  PDW- 10-14 fl

  PCT- 0.18-0.34%

  P-LCR -11-33%

  Hematocrit- 36-46%

  Total leucocyte count-4000-11000 cells/cmm

INCLUSION CRITERIA:

- All the patients with serologically positive dengue infection were included.

EXCLUSION CRITERIA:

- Positive dengue patients with diabetes mellitus, hypertension, aplastic anemia, leukemias, patients on chemotherapeutic drugs, pregnancy and other co infections like malaria, typhoid were excluded from the study.

METHOD OF COLLECTION OF DATA:

All the blood samples of cases satisfying the inclusion and exclusion criteria were collected in k2 EDTA vacutainers and were processed on Sysmex XN1000 haematology analyser which is based on the principle of fluorescence flow cytometry.

SAMPLE SIZE:

With Mean (SD) of platelet count 25(5) at 99% confidence interval and 5% margin of error, sample size is 106 using the formula[4]

$$n = Z^2 \times SD^2 / d^2$$

where Z = Z value at α level =95%

SD = standard deviation

d = Margin of error

In the present study 123 cases were studied

STATISTICAL ANALYSIS:

Data was analysed using**:**

- Mean ± SD
- Students ‘t’ test / Mann Whitney ‘U’ test
- ANOVA test
- Chi-square test

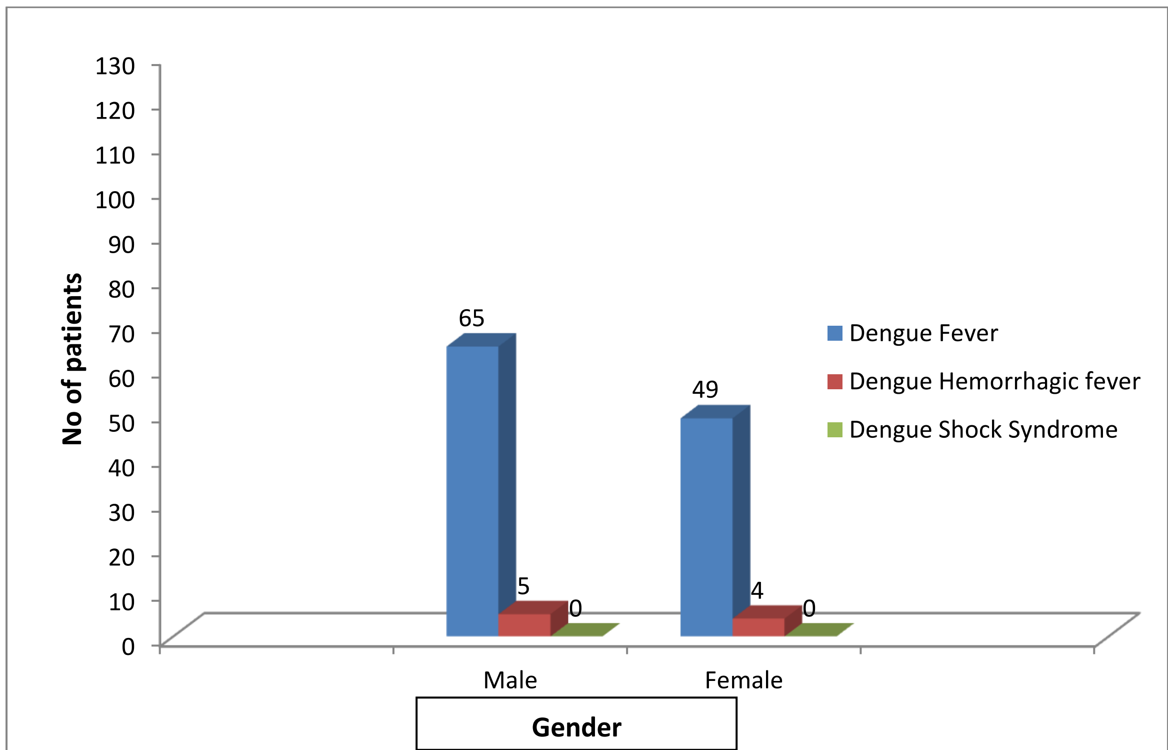
RESULTS

One hundred and twenty three patients, diagnosed with dengue infection based on ELISA NS1, IgM, IgG positivity were included in the study.

TABLE 3:- DISTRIBUTION OF CASES ACCORDING TO GENDER

Gender	Dengue Fever		Dengue Hemorrhagic Fever		Dengue shock
	No.of patients	percentage	No. of patients	percentage	
Male	65	57.0	5	55.6	00
Female	49	43.0	4	44.4	00
Total	114	100.0	9	100	00

FIGURE 8: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO GENDER

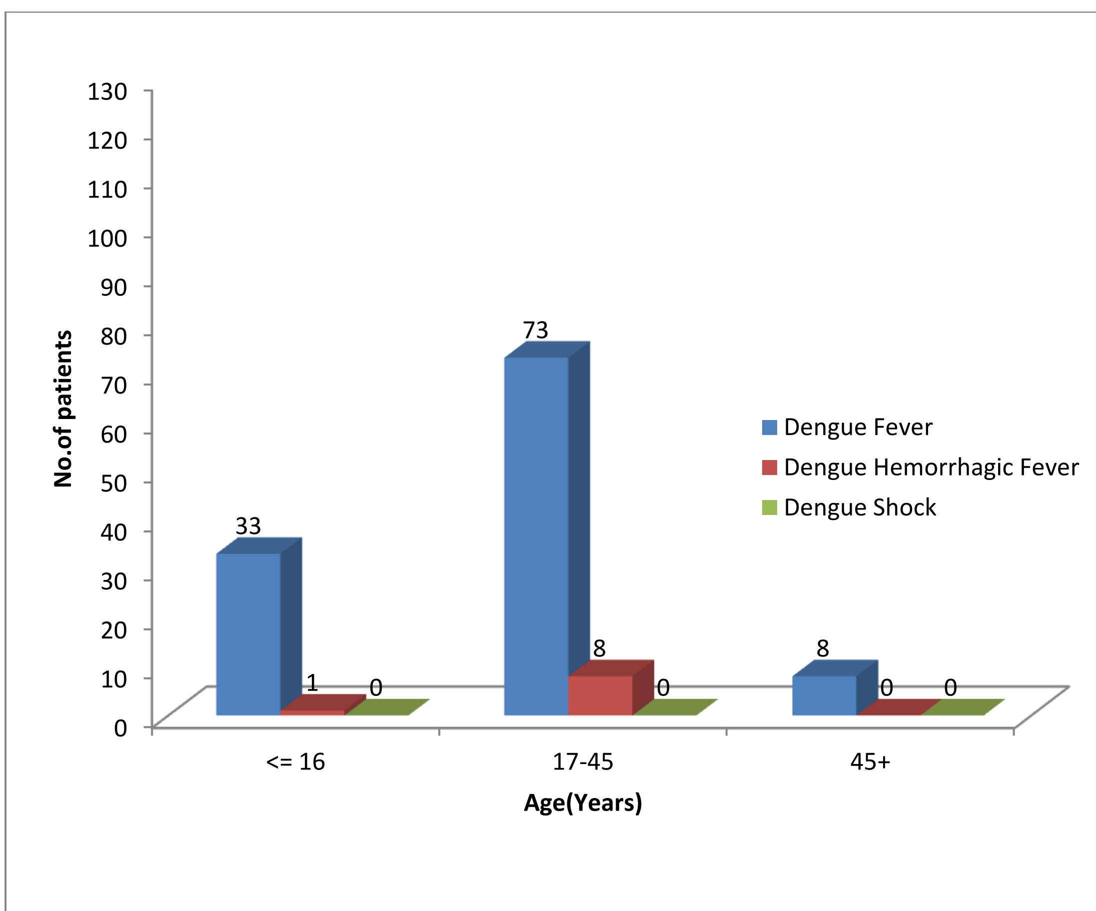


In the present study ratio of M:F in DF is 1.32:1 and in DHF it is 1.25:1.

TABLE 4: DISTRIBUTION OF CASES BY AGE

Age (Years)	Dengue Fever		Dengue Hemorrhagic Fever		Dengue Shock Syndrome
	No. of patients	percentage	No. of patients	percentage	
≤ 16	33	29	1	11	00
17-45	73	64	8	89	00
45+	8	7	0	0	00
Total	114	100.0	9	100.0	00

FIGURE 9: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO AGE



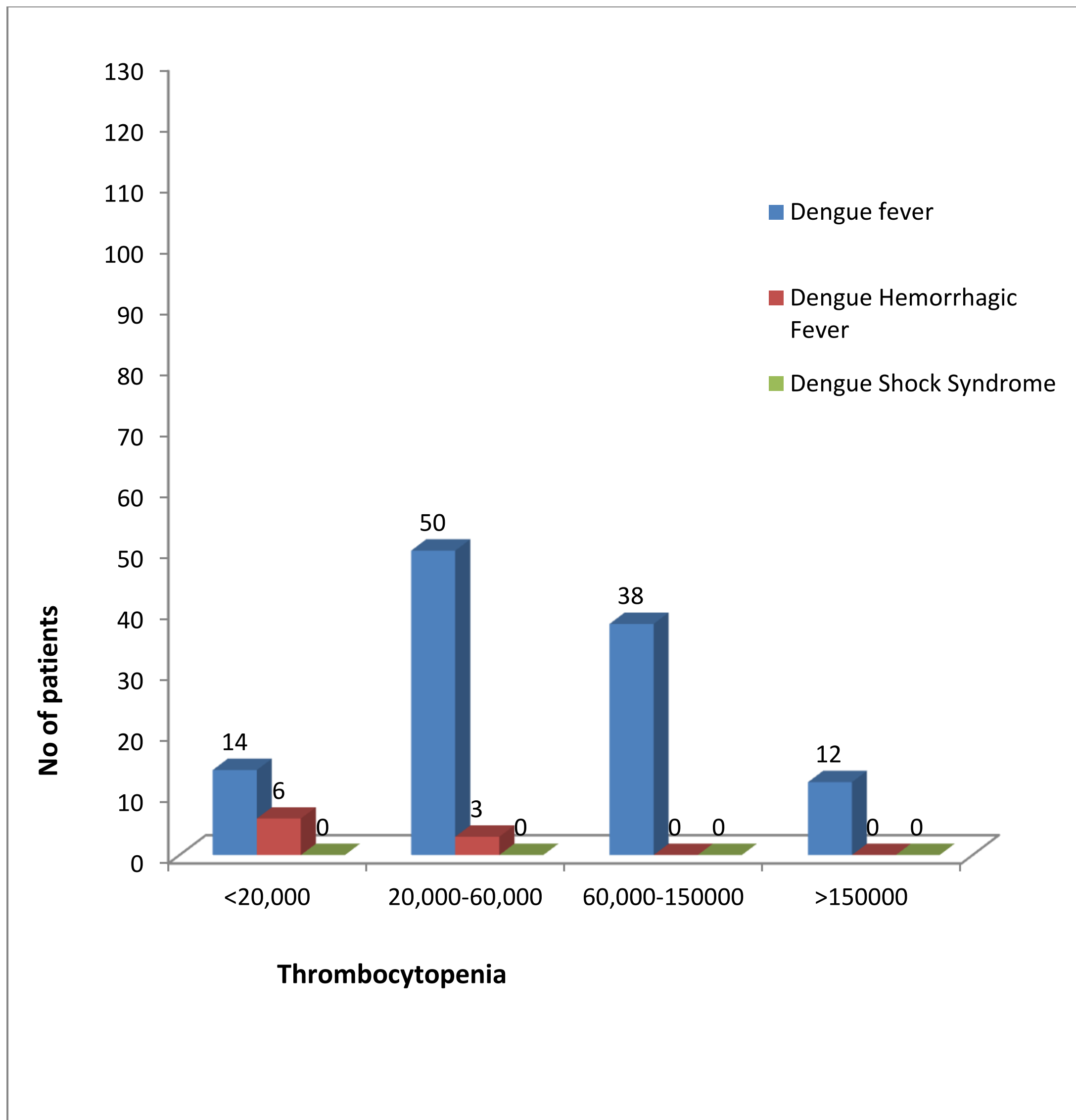
In the present study age group of dengue infection ranged from 2-75years. Majority of the patients were in between the age group 17-45 years in both DF 73(64%) and DHF 8(89%).

HEMATOLOGICAL FINDINGS

TABLE 5: DISTRIBUTION OF PATIENTS ACCORDING TO PLATELET COUNT

Platelet count	Dengue Fever		Dengue Hemorrhagic Fever		Dengue Shock syndrome	Chi square test
	No.of patients	Percentage	No.of patients	Percentage	No.of patients	
< 20000 (severe)	14	12.28	6	66.66	00	p=0.0007*
20000-60000 (moderate)	50	43.86	3	33.34	00	
60000-150000 (mild)	38	33.33	0	0	00	
>150000 (normal)	12	10.53	0	0	00	
Total	114	100.0	9	100.0	00	

FIGURE 10: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO PLATELET COUNT

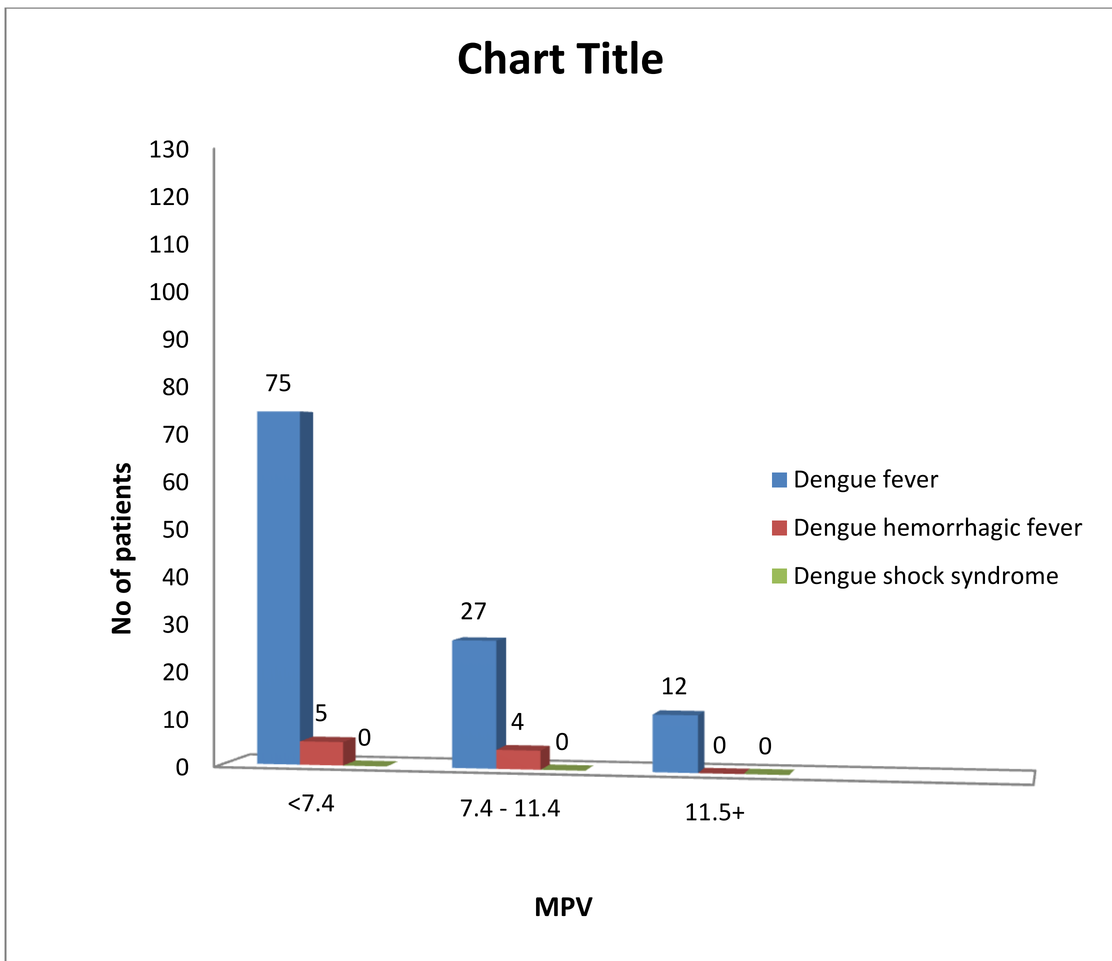


111 cases out of total 123 cases amounting to 90.2% had thrombocytopenia in the present study. Out of 114 cases of DF 50 (43.86%) had moderate thrombocytopenia whereas out of 9 cases of DHF 6(66.66%) had severe thrombocytopenia. A p value of 0.0007 was found to be significant among the two groups.

TABLE 6:- DISTRIBUTION OF PATIENTS ACCORDING TO MPV

MPV	Dengue Fever		Dengue Hemorrhagic Fever		Dengue Shock syndrome	Chi square test
	No.of patients	Percentage	No.of patients	Percentage	No. of patients	
< 7.4(Low)	75	65.78	5	55.55	00	p=0.2843 NS
7.4 - 11.4 (Normal)	27	23.68	4	44.45	00	
11.5+(High)	12	10.54	0	0.0	00	
Total	114	100.0	9	100.0	00	

FIGURE 11: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO MPV

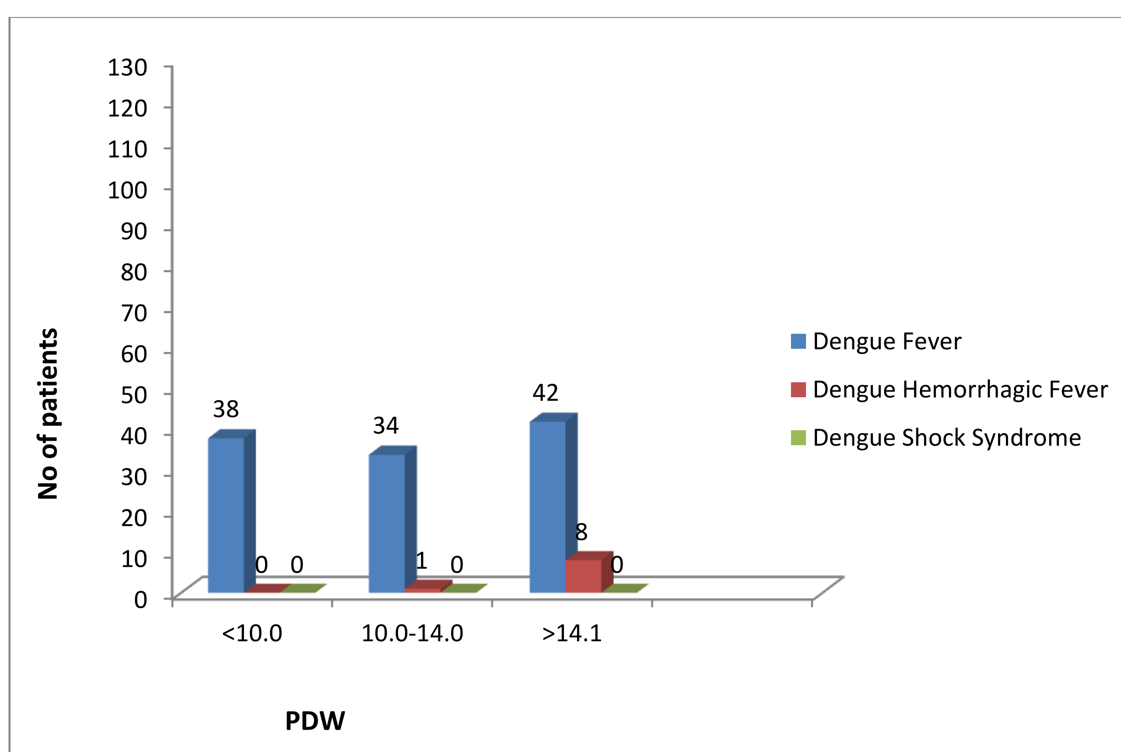


In the present study 80 out of 123 cases showed low MPV amounting to 65.04% of the cases. Out of 114 DF cases, low MPV was noted in 75(65.78%) and in 9 cases of DHF low MPV was noted in 5(55.55%). In the present study p value was 0.2843 and its was not significant among the two groups.

TABLE 7:- DISTRIBUTION OF PATIENTS ACCORDING TO PDW

PDW	Dengue Fever		Dengue Hemorrhagic Fever		Dengue Shock syndrome	Chisquare test
	No.of patients	Percentage	No. of patients	Percentage	No.of patients	p=0.03*
< 10.0(Low)	38	33.34	0	0	00	
10.0- 14.0 (Normal)	34	29.82	1	11.11	00	
14.1+(High)	42	36.84	8	88.89	00	
Total	114	100.0	9	100.0	00	

FIGURE 12: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO PDW

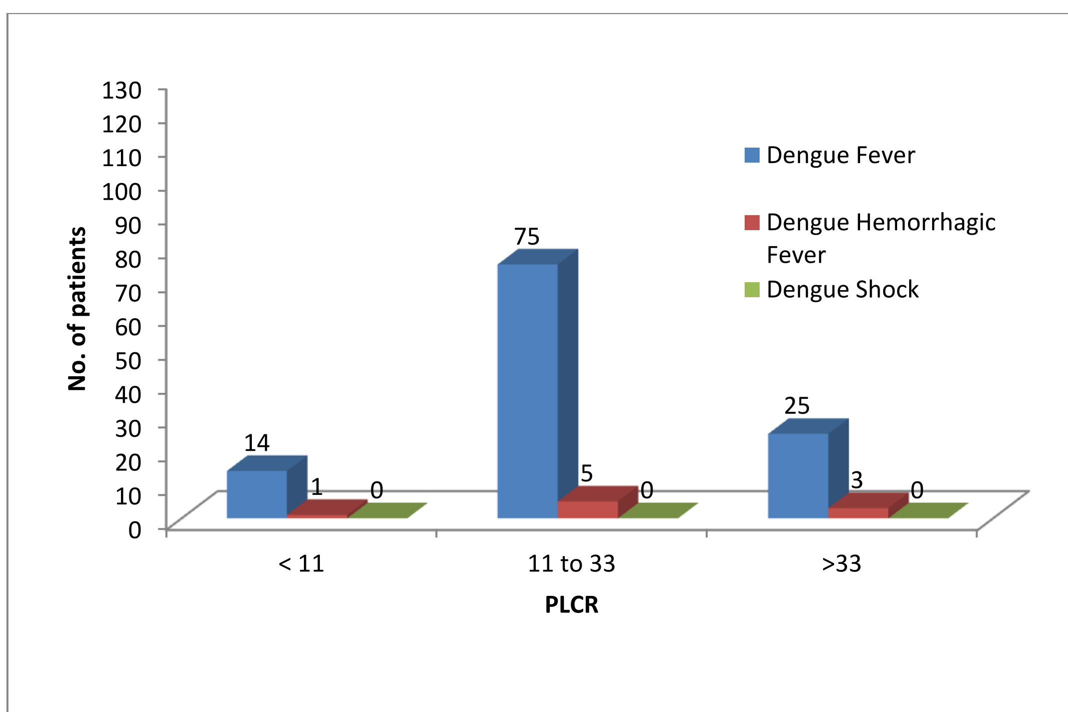


In the present study 50 out of 123 dengue infection cases that is 40.65% had high PDW. Amongst them high PDW was noted in 42(36.84 %) cases of DF and in 8(88.89%) cases of DHF. In the present study p value was 0.03 and its was found to be significant among the two groups.

TABLE 8:- DISTRIBUTION OF PATIENTS ACCORDING TO PLCR

PLCR	Dengue Fever		Dengue Hemorrhagic Fever		Dengue shock syndrome	Chisquare test
	No. of patients	percentage	No. of patients	percentage	No. of patients	
<11(Low)	25	21.93	3	33.33	00	p =1.001 NS
11-33 (Normal)	75	65.79	5	55.55	00	
>33(High)	14	12.28	1	10.12	00	
Total	114	100	9	100.0	00	

FIGURE 13: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO PLCR

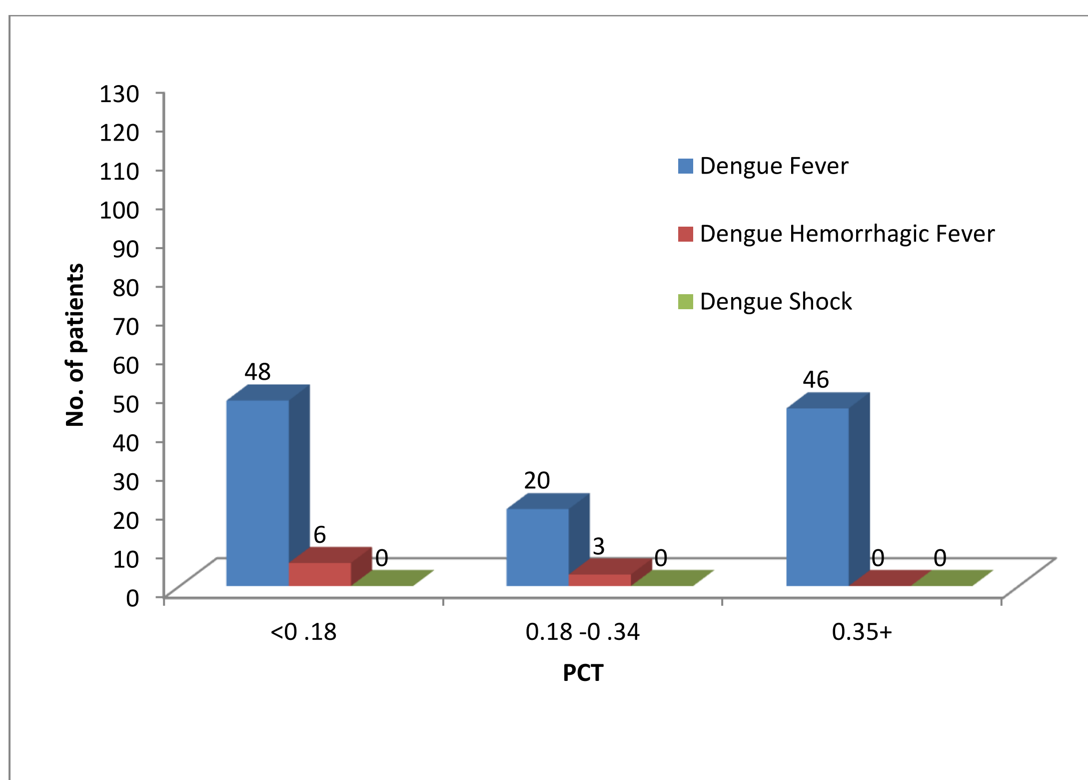


In the present study out of 123 cases of dengue infection 80 cases had normal value of P-LCR amounting to 65.04%. Amongst them normal P-LCR values were noted in 75(65.79%) of DF and 5(55.55%) of DHF. A P Value of 1.001 was found in our study, which is not significant.

TABLE 9:- DISTRIBUTION OF PATIENTS ACCORDING TO PCT

PCT	Dengue Fever		Dengue Hemorrhagic Fever		Dengue shock syndrome	Chi Square Test
	No.of patients	Percentage	No.of patients	Percentage	No.of patients	
< .18(Low)	48	42.1	6	66.67	00	p=0.0426*
.18 - .34 (Normal)	20	17.5	3	33.33	00	
.35+(High)	46	40.4	0	0	00	
Total	114	100.0	9	100.0	00	

FIGURE 14: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO PCT

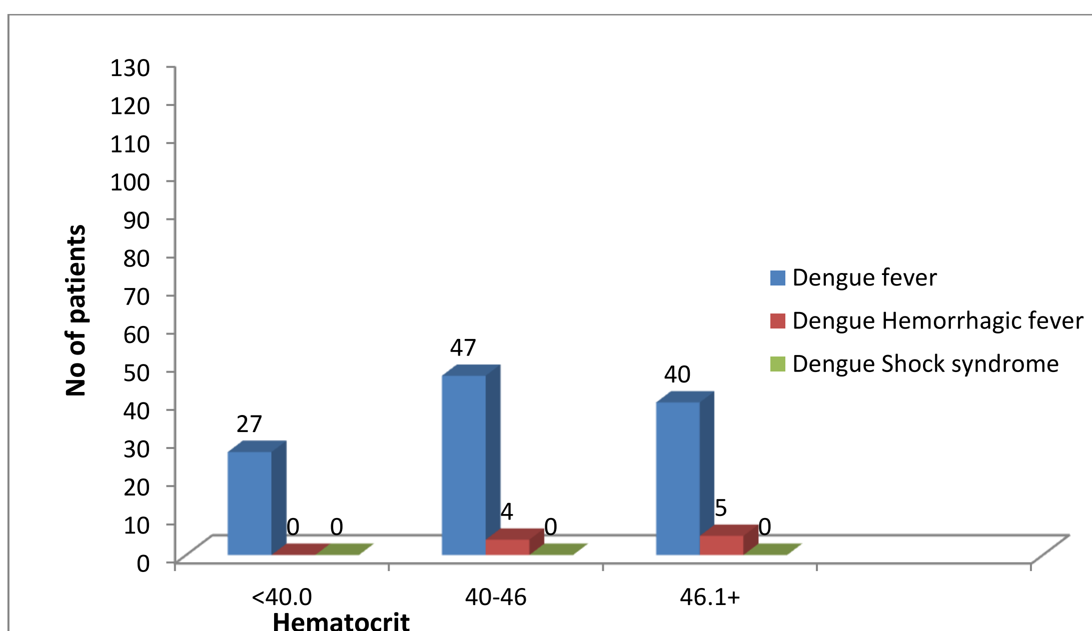


In the present study 54 out of 123 cases amounting to 43.09% showed low PCT in cases of dengue infection Amongst them. 48(42.1%) had low PCT in DF and 6(66.67%) cases had low PCT in DHF. p value of 0.0426 was found in the present study which is significant.

TABLE 10:- DISTRIBUTION OF PATIENTS ACCORDING TO HEMATOCRIT

Hematocrit	Dengue Fever		Dengue Hemorrhagic Fever		Dengue shock syndrome	Chi square test
	No.of patients	Percentage	No.of patients	Percentage	No.of patients	
< 40.0(Low)	27	23.68	0	0	00	p=0.1063 NS
40.0 - 46.0 (Normal)	47	41.24	4	44.45	00	
46.1+(High)	40	35.08	5	55.55	00	
Total	114	100.0	9	100.0	00	

FIGURE 15: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO HEMATOCRIT

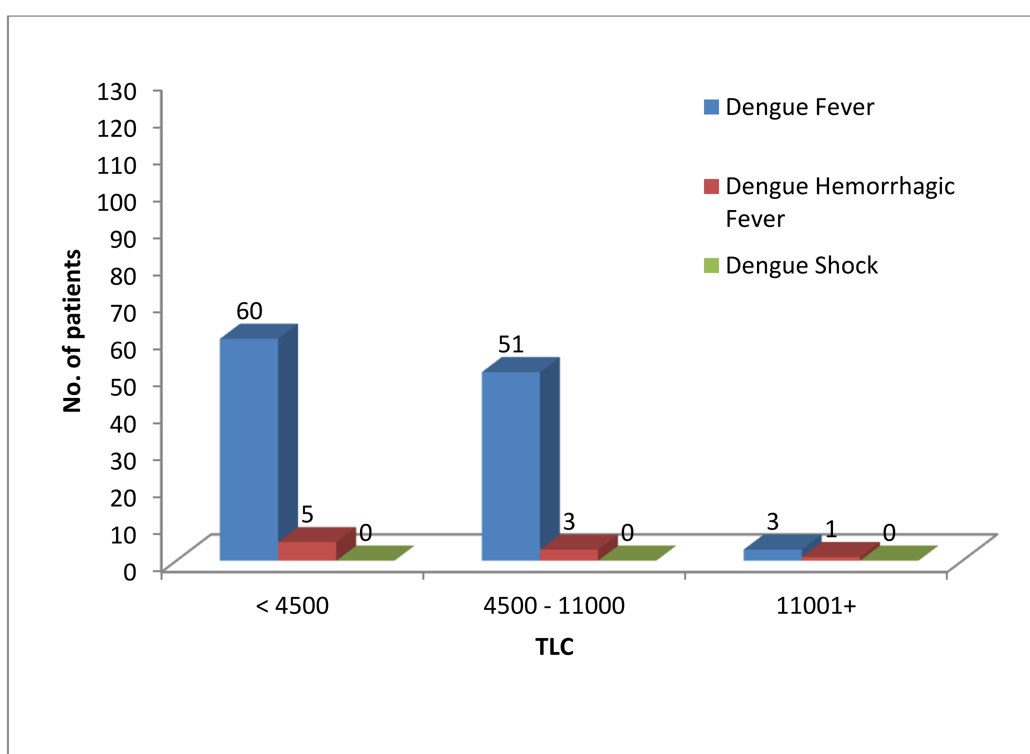


In the present study 45 out of 123 dengue infection cases amounting to 36.58% had high hematocrit. Amongst them 40(35.08%) of cases of DF and 5(55.55%) cases of DHF had high hematocrit. A p value of 0.1063 was found not to be significant among the two groups.

TABLE 11 :- DISTRIBUTION OF PATIENTS ACCORDING TO TLC

TLC	Dengue Fever		Dengue Hemorrhagic Fever		Dengue shock syndrome	Chi square test
	No.of patients	Percentage	No.of patients	Percentage	No.of patients	
< 4500(Low)	60	52.6	5	55.55	00	p=0.15 NS
4500-11000 (Normal)	51	44.7	3	33.34	00	
11001+(High)	3	2.7	1	11.11	00	
Total	114	100.0	9	100.0	00	

FIGURE 16: BAR GRAPH SHOWING DISTRIBUTION OF CASES ACCORDING TO TLC

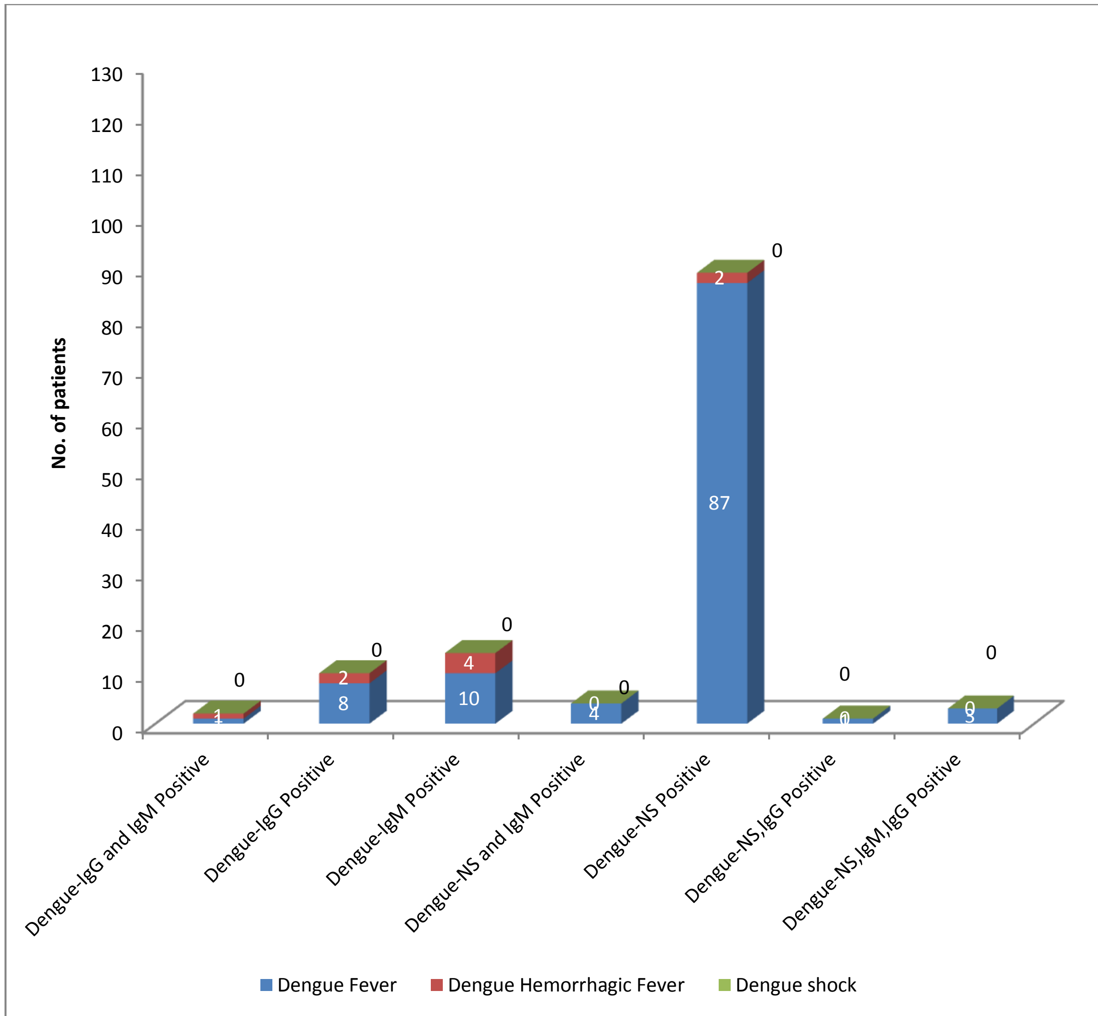


In the present study 65 out of 123 cases of dengue infection had low leucocyte count amounting to 52.84%. Amongst them 60(52.6%) of DF cases and 5(55.55%) cases of DHF had low leucocyte count. A p value of 0.15 was found in the present study which is not significant.

TABLE 12:- DISTRIBUTION OF PATIENTS ACCORDING TO DENGUE SEROLOGY POSITIVITY

Dengue Serology	Dengue Fever		Dengue Hemorrhagic Fever	percentage
	No. of patients	percentage	No. of patients	
Dengue-IgG and IgM Positive	1	0.9	1	11.1
Dengue-IgG Positive	8	7.0	2	22.2
Dengue-IgM Positive	10	8.8	4	44.5
Dengue-NS and IgM Positive	4	3.59	0	0
Dengue-NS Positive	87	76.31	2	22.2
Dengue-NS, IgG Positive	1	0.9	0	0
Dengue-NS,IgM,IgG Positive	3	2.5	0	0
Total	114	100.0	9	100.0

FIGURE 17: BAR GRAPH SHOWING DISTRIBUTION OF PATIENTS ACCORDING TO DENGUE SEROLOGY POSITIVITY IN DENGUE INFECTION



Majority of patients in DF were NS1 positive 87(71.4%), while in DHF majority were 4(44.4%) Dengue IgM positive.

TABLE 13:- COMPARISON BETWEEN DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER

Variables	Dengue Fever		Dengue Hemohhagic fever		Unpairedt test/Mannwhitney test
	Mean	SD	Mean	SD	
Platelet count	0.9155	0.52470	0.194	0.128	p=0.0001*
MPV	9.69	1.83	8.63	2.56	p=0.1999NS
PDW	12.91	2.91	14.71	1.13	p=0.0396*
PLCR	26.98	8.76	22.54	7.81	p=0.1635 NS
PCT	0.218	0.099	0.142	0.89	p=0.0319*
Hematocrit	40.78	5.95	44.73	2.41	p=0.0670NS
TLC	4675.94	2217.04	4710	3079.7	p=0.624 NS

*Indicated significant difference; NS Indicates No significant difference

Platelet count, PDW and Plateletcrit showed significant differences ($P<0.05$) among the two groups i.e Dengue fever and Dengue Hemorrhagic Fever

DISCUSSION

Dengue infection has become a major health issue affecting tropical and sub-tropical regions around the world – especially urban, peri-urban and rural areas. Dengue has a world wide distribution and remains a health problem or potential threat in areas infested with Aedes aegypti mosquito.[54]

Dengue infection is the most rapidly spreading mosquito borne viral disease in the world. In dengue infection clinical illness ranges from inapparent or mild febrile illness such as dengue fever (DF) to its complications such as dengue haemorrhagic fever(DHF) and dengue shock syndrome (DSS). Dengue Fever is the most common manifestation of dengue infection as it is a self limiting disease. The present strategy is to control infection and prevent complications.[55]

In the present study emphasis was laid upon the utility of total leucocyte count, hematocrit and platelet indices in accessing the severity of dengue infection.

TABLE 14: COMPARISION OF GENDER WISE DISTRIBUTION IN DENGUE INFECTION WITH OTHER STUDIES

S.No	Author	Place	Ratio of Males: Females
1	Neerja M *et al*[56]	Hyderabad	2:1
2	Meena CK *et al*[45]	Rajasthan	1.7:1
3	Shekar GC *et al*[57]	Warangal	1.13:1
4	**Present Study**	**Vijayapura**	**1.32:1**

The present study male to female ratio was 1.32:1 in Dengue Infection.

A study done by Neeraja M *et al*[56] and Meena CK *et al*[45] had more male prevalence in their study and this could be due to the more outdoor life of males than females.

Table 15: COMPARISION OF CLINICAL PROFILE OF THE DENGUE INFECTION WITH OTHER STUDIES

S.NO	AUTHOR	YEAR	PLACE	Clinical spectrum (in %)		
				DF	DHF	DSS
1	Pancharoen *et al*[38]	1995	Thailand	22.3	60.4	17.3
2	Neeraja M *et al*[56]	2004	Hyderabad	85	5	10
3	Shekar GC *et al*[57]	2013	Telangana	81	10	9
4	Meena CK *et al*[45]	2016	Rajasthan	84	14	2
5	Shams N *et al*[52]	2016	Pakisthan	75	23	2
6	**Present Study**	**2018**	**Vijayapura**	**92**	**8**	**0**

Out of 123 serologically proven cases of dengue infection studied, 114 (92%) cases were of dengue fever and 9(8%) cases were of dengue hemorrhagic fever. There were no cases of Dengue Shock Syndrome included in the present study. This is in accordance with the study done by Neeraja M *et al*[56], Shekar GC *et al*[57] showing that majority of the patients had simple Dengue fever and fewer developed DHF and DSS which is due to more awareness about the health care among the patients leading to early visit to the hospital.

A study done by Pancharoen *et al* [38] had more number of DHF in their study this was due to large outbreaks of dengue infection in Thailand in 1987 and is also believed

that dengue virus type 3, possibly the most neurovirulent type of dengue virus, was dramatically more common during these outbreaks. Also from these observations, we can conclude that the incidence of each clinical spectrum varies with geographical area.[57]

HEMATOLOGICAL PARAMETERS

TABLE 16: COMPARISION OF THROMBOCYTOPENIA IN DF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with Thrombocytopenia
1	Cherian T *et al* [58]	Hyderabad	94.7%
3	Khan E *et al* [59]	Thailand	81.4%
5	Meen CK *et al*[45]	Rajasthan	90%
7	Gajera VV*et al*[6]	Navi Mumbai	81%
8	Suva C *et al*[60]	Jamnagar	79%
10	Lokantha H*et al*[47]	Bengaluru	85.9%
11	Yashaswini LS *et al*[61]	Bengaluru	89%
13	**Present study**	**Vijayapura**	**89.47%**

Thrombocytopenia is considered to be one of the major criteria utilized by WHO rules as a potential marker of clinical severity.[62] In the present study thrombocytopenia was observed in 89.47% cases of DF and this was in correlation with the above mentioned studies.

TABLE 17: COMPARISION OF THROMBOCYTOPENIA IN DHF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with Thrombocytopenia
1	Shams N *et al*[52]	Pakisthan	96%
2	Sivathanu S *et al*[63]	Chennai	100%
3	Present study	Vijayapura	100%

In present study thrombocytopenia was noted in 100% of DHF cases. Similar results were noted in studies conducted by Shams N *et al*[52] and Sivathanu S *et al*[62]. Explanation for thrombocytopenia being present in more number of cases of DHF in comparision to DF was given by Joshi AA[44]. He observed that most of the cases of DHF and DSS in their study had an antibody only pattern more than NS1 antigen pattern. This was also in concordance with few other studies which claimed increased association of thrombocytopenia with antibody patterns.

P value in the present study was 0.0007. This is in concordance with similar studies conducted by Shams N *et al*[52], Nehara H R *et al*[3] and Rathod N P *et al*[34]. Thus low platelet count is a predictor of severity of dengue infection.

TABLE 18: COMPARISION OF MPV, PDW IN DF WITH OTHER STUDIES

S. NO	Author	MPV			PDW		
		Low (%)	**Normal (%)**	**High (%)**	**Low (%)**	**Normal (%)**	**High (%)**
1	Navya *et al*[53]	68.8%	-	31.1%	6.66%	-	93.33%
2	Nehera RH *et al*[3]	71.9%	28.05%	-	-	26.83%	73.17%
3	**Present Study**	**65.78%**	**23.68%**	**10.54%**	**33.34%**	**29.82%**	**36.84%**

TABLE 19: COMPARISION OF MPV, PDW IN DHF WITH OTHER STUDIES

S.NO	Author	MPV			PDW		
		Low (%)	**Normal (%)**	**High (%)**	**Low (%)**	**Normal (%)**	**High (%)**
1	Navya *et al*[53]	100%	-	00%	20%	-	80%
2	Nehera RH *et al*[3]	61.11%	38.89%	00%	00%	00%	100%
3	**Present Study**	**55.55%**	**44.45%**	**00%**	**00%**	**11.11%**	**88.89%**

To our knowledge there are not many studies which have investigated changes in platelet indices during dengue infection. A low MPV suggests marrow suppression as a cause of thrombocytopenia and a rising MPV marks the beginning in the improvement in platelet count.[64]

The activation of platelets leads to morphological alterations by attaining spherical shape and formation of pseudopodia making it larger. Thus platelets with pseudopodia will increase in size leading to rise in PDW. [65]

In the present study MPV was low and PDW was high in majority of the cases of DF and DHF. This finding is in concordance with studies conducted by Navya *et al*[53] and Nehera RH *et al*[3] as mentioned in the above table.

In our study p value for MPV was 0.2843 which proves that there is no significant difference in DF and DHF. A study conducted by Sharma K *et al*[64] and Dewi *et al*[66] also observed that MPV showed no significant correlation with severity, serology and treatment outcome of dengue infection. Thus excluding the role of MPV in comparing the severity between DF and DHF cases.

In the present study p value for PDW was 0.03 which was found to be significant. This is in concordance with studies conducted by Navya *et al*[53] and Nehera RH *et al*[3]. This proves its role in predicting the severity of dengue infection.

TABLE 20: COMPARISION OF PLATELETCRIT IN DENGUE INFECTION WITH OTHER STUDIES

S.NO	Author	DF(mean)	DHF(mean)	P value
1	Nehera RH *et al*[3]	0.0482± 0.0259	0.0328±0.0148	<0.0166*
2	**Present Study**	**0.218±0.099**	**0.142±0.89**	**0.0319***

* indicates significant difference.

In our study p value for plateletcrit shows significant difference which is in concordance with studies undertaken by authors like Nehera HR *et al*[3] and Mukker P[62] *et al*. Hence, Plateletcrit can be used as a predictor of severity and outcome of dengue infections.

TABLE 21: COMPARISION OF PLCR IN DENGUE INFECTION WITH OTHER STUDIES

S.NO	Author	DF(mean)	DHF(mean)	P value
1	Nehera RH *et al*[3]	43.79± 7.131	44.72±2.829	<0.5913
2	**Present Study**	**26.98±8.76**	**22.54±7.81**	**0.1635 NS**

NS indicates no significant difference

In our study p value for PLCR shows no significant difference which is in concordance with study conducted by authors like Nehera HR *et al*[3] and Bashir P *et al*[5]. Hence, PLCR cannot be used as a predictor of severity and outcome of dengue infections.

Thus, Low platelet count, low PCT and high PDW can be used as predictors of severity of Dengue infection.

TABLE 22: COMPARISION OF HEMATOCRIT IN DF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with Increased Hematocrit
1	Kalayanarooj S *et al*[67]	Thai	18%
2	Mia MW *et al*[68]	Bangladesh	27%
3	Vulavala S *et al*[41]	Kolar	23.22%
4	Gajera VV *et al*[6]	Mumbai	28%
5	Rathod PN *et al*[34]	Mumbai	41%
6	**Present study**	**Vijayapura**	**35.08%**

TABLE 23: COMPARISION OF HEMATOCRIT IN DHF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with Increased Hematocrit
1	**Lokanatha H *et al*[47]**	Bengaluru	68.2%
2	**Present study**	**Vijayapura**	**55.55%**

The raised hematocrit is considered as an accurate indicator of vascular permeability and plasma leakage.[47]

Many studies have been done on the hematological profile of Dengue and found that raised hematocrit is one of the well known findings in patients of Dengue infection.

In the present study a significant difference was not observed in hematocrit among the two groups (p=0.1063).

Similar findings were noted in the study conducted by Lokanatha H *et al*[47]. He also suggested that not much significant difference was observed between the raised hematocrit in dengue fever and dengue hemorrhagic fever.

TABLE 24: COMPARISION OF TOTAL LEUCOCYTE COUNT IN DF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with leucopenia
1	Meena CK *et al*[45]	Rajasthan	51%
2	Suva C *et al*[60]	Jamnagar	56%
3	Yashaswini LS *et al*[61]	Bengaluru	52%
4	Moideen JCA *et al*[69]	Manglore	>50%
5	**Present study**	**Vijayapura**	**52.6%**

TABLE 25: COMPARISION OF TOTAL LEUCOCYTE COUNT IN DHF WITH OTHER STUDIES

S.NO	AUTHOR	PLACE	% of cases with leucopenia
1	Shams N *et al*[45]	Pakisthan	75%
2	**Present study**	**Vijayapura**	**55.55%**

Leucopenia is also a major findings in dengue fever. A number of studies have shown that patients presenting with either primary or secondary infections during their early phases of infection manifests a decline in leucocyte count and this finding corresponds to the suppression of marrow during acute phase.[2]

In the present study leucopenia was noted in both DF and DHF. A significant change in leucopenia was not observed among the two groups. Many studies done by authors like Sivathanu S *et al*[63] and Loknatha H *et al* [47] also observed that the there was no association of leucopenia with severity of Dengue infection.

SUMMARY

- This study was undertaken in B.L.D.E. (Deemed to be University) Shri B. M. Patil Medical College, Hospital and Research center, Vijayapura, Karnataka to study the efficacy of total leucocyte count, hematocrit and platelet indices in predicting the severity of dengue infection.
- Age group of all the Dengue Infection patients ranged from 2-75 years.
- A total of 123 dengue infection cases were studied, out of which 114 were of dengue fever and 9 were of dengue hemorrhagic fever, there were no cases of dengue shock syndrome.
- Among the haematological parameters thrombocytopenia was most common finding present in 89.47% cases of DF and 100% cases of DHF
- A low PCT was found in 66.67% cases of DHF and 42.1% cases of DF. A significant P value of 0.426 was observed.
- High PDW was observed in 36.84% of DF and 88.89% cases of DHF cases with a significant P value of 0.03.
- There was a significantly lower platelet count, Lower PCT and higher PDW in DHF as compared to DF.
- It was also observed that there was raised hematocrit in dengue infection and was found more in DHF (55.56%) as compared to DF (35.08%) cases .
- Leucopenia was observed in 52.6% cases of DF and 55.55% cases of DHF.
- Raised hematocrit and leucopenia were also consistent findings in Dengue infection and thus can aid in the diagnosis

CONCLUSION

- Platelet indices play an important role in diagnosis of dengue infection and can be used in predicting the severity of dengue viral infection. Hemoconcentration and leucopenia are also consistent findings in Dengue infection
- Since DF does not have a specific medical therapy, clinical recovery monitoring is largely dependent on hematological parameters. Utilizing these predictive variables, severe dengue may be identified and with careful monitoring, we may reduce complications and mortality in high risk cases. Thus, these parameters can be used as screening tools by physicians to chart early therapeutic response.

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ANNEXURES

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04/10/2016 *at* 3-00pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title Utility of leucocyte count, hematocrit and platelet indices in predicting the severity of dengue infection

Name of P.G. student Moksha. S
dept of pathology

Name of Guide/Co-investigator Dr S.B. Hipparagi
professor in pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,________________ ,S/O or D/O _________________,aged____ years, ordinarily resident of _____________do hereby state/declare that Dr. ____________________ of Hospital has examined me thoroughly on _______________ at _______________ (place) and it has been explained to me in my own language that I am suffering from _________________ disease (condition) and this disease/condition mimic following diseases . Further Doctor informed me that he/she is conducting dissertation/research titled ___________________under the guidance of Dr ________________requesting my participation in the study. Apart from routine treatment procedure, the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Doctor has also informed me that during conduct of this procedure like adverse results may be encountered. Among the above complications most of them are treatable but are not anticipated hence there is chance of aggravation of my condition and in rare circumstances it may prove fatal in spite of anticipated diagnosis and best treatment made available. Further Doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt ______________________________ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place

PROFORMA FOR STUDY :

Demographic Details:

Name :

Age :

Sex : M/F

RELIGION :

OCCUPATION :

RESIDENCE :

OPD / IPD No. :

Lab. No. /Sample No. :

Chief complaints:

History of present illness:

Past history:

Family history:

General physical examination:

Systemic examination:

- **Cardiovascular system**
- **Respiratory system**
- **Central Nervous System**
- **Per Abdomen Examination**

Clinical diagnosis:

Hematological investigations:

Parameters	
MPV	
PDW	
P-LCR	
PCT	
PLATELET COUNT	
HEMATOCRIT	
TOTAL LEUCOCYTE COUNT	

KEY TO MASTER CHART

Y	Years
M	Male
F	Female
PLT	Platelet Count
MPV	Mean Platelet Count
PDW	Platelet Distribution Width
P-LCR	Platelet large cell ratio
PCT	Plateletcrit
TLC	Total Leucocyte count