

## Original Research Article

# Study of haematological, biochemical profile and clinical presentation in dengue positive patients: 82 cases

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## ABSTRACT

**Background:** Dengue Fever (DF) is a self-limiting disease caused by arbovirus and transmitted by *Aedes* mosquitoes (*Aedes aegypti* and *Aedes albopictus*). It is one of the 17 neglected tropical diseases by WHO. Diagnosis of dengue depends mainly on the detection of IgM and IgG antibody, and NS1 antigen.

**Methods:** The study was carried out in Department of Pathology, affiliated with a government hospital. It includes 82 dengue patients, admitted from August 2015 to August 2016. Haematological, biochemical profile, clinical signs and symptoms were recorded. The Tourniquet test was performed in all the patients on admission. Grading of dengue: DF/DHFI/DHFII/DHFIII/DHFIV. Grade III and IV were collectively called as Dengue Shock Syndrome.

**Results:** Total 82 Dengue positive cases were studied, 52 (63%) were males and 30 (37%) were females. 24 (29%) patients were recorded in September 22 (27%) in October 19 (23%) in August. 12 (14.60%) had positive tourniquet test. Thrombocytopenia was present in 86.5 % patients. Majority cases were of classical dengue fever 51 (62.20%), 14 (17.07%) were of DHF I, 12 (14.63%) were of DHF II, 3 (3.66%) were of DHF III and 2 (2.44%) were of DHF IV.

**Conclusions:** It is very important to correlate clinical examination with haematological and biochemical profile in dengue patients. Hematocrit value, leucopenia, thrombocytopenia, raised liver enzymes is very important to monitor dengue cases in their initial stages and thus facilitate early treatment. This would minimize morbidity and mortality arising out of serious complications of dengue fever.

**Keywords:** Dengue, DHF, Grading, DSS, Vector borne disease

## INTRODUCTION

Dengue is a self-limiting acute mosquito transmitted disease characterized by fever, headache, muscle and joint pains, rash, nausea and vomiting. Dengue fever (DF) is caused by an arbovirus and spread by *Aedes* mosquitoes. Some infections result in Dengue Hemorrhagic Fever (DHF) and in its severe form Dengue Shock Syndrome (DSS). Over the past two decades, there has been global increase in the frequency of DF, DHF and its epidemics. Various factors responsible for the resurgence of dengue epidemic are: human population growth, un-planned and un-controlled urbanization, inadequate waste management, water supply

mismanagement, increased distribution and densities of vector mosquitoes, lack of effective mosquito control has increased movement and spread of dengue viruses and development of hyper-endemicity.<sup>1</sup> Dengue has been identified as one of the 17 neglected tropical diseases by WHO (World Health Organization) as mentioned in their first report on neglected tropical diseases (2010). Of the 11 countries of SEAR, 10 countries including India are endemic for dengue. The disease has a seasonal pattern and the cases peak after the monsoons.<sup>2</sup> There are 3 epidemiological factors: the host (man and mosquito), The agent (virus), The environment (abiotic and biotic factors) agent for dengue viruses belong to the genus *Flavivirus*. The virion comprises a spherical particle, 40-

50 nm in diameter, with a lipopolysaccharide envelope. The positive single-strand RNA genome, which is approximately 11 kb in length, has a single open reading frame that encodes three structural proteins: Capsid (C), Membrane (M) glycoproteins, Envelope (E) glycoproteins, seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). There are four serotypes of the Dengue virus referred to as: DV-1, DV-2, DV-3, DV-4.<sup>1-3</sup> Dengue viruses are transmitted from an infected person to others by the bite of the female mosquito (Vector).

In India, *Aedes aegypti* and *Aedes albopictus* are the main vectors. Other species like *Aedes polynesiensis* and *Aedes neivus* have also been incriminated as secondary vectors in some countries.<sup>4</sup> *Aedes aegypti* is a primary vector of viral diseases such as the dengue fever, chikungunya viruses and yellow fever. *Aedes albopictus* is also called the Asian tiger mosquito and is most well-known for transmitting dengue and chikungunya viruses, west Nile, Eastern equine encephalitis, Japanese encephalitis. Environmental factor- *Aedes Aegypti* breeds almost entirely in domestic man-made water receptacles found in and around households water storage containers, water reservoirs, overhead tanks, desert coolers, tyres, coconut shells, unused grinding stones etc. Natural larval habitats include tree holes, latex collecting cups in rubber plantations, bamboo stumps etc.

**Host factor-** The dengue virus infects humans and several species of lower primates. Travel to dengue endemic areas is a most important risk factor. The virus is transmitted when the infected female mosquito bites and injects its saliva into the wound of the person bitten. Dengue transmission can also occur through blood transfusion, organ transplantation, congenital dengue infections in neonates born to infected mothers.<sup>4</sup> Primary infection is infection caused by any serotype in non-immune individual. Secondary infection is heterotypic infection in a monotypic immune individual.

Primary infection is usually benign in nature, however secondary infection with a different serotype or multiple infections with different serotypes may cause severe infection that can be classified as either dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS). Primary infections are characterized by an increase in dengue-specific IgM antibodies four to five days after the onset of fever and by an increase in IgG antibodies only after the onset of fever and by an increase in IgG antibodies only after seven to ten days. IgM antibodies are detectable for three to six months, whereas IgG antibodies remain detectable for life. In secondary infections, the level of IgM antibodies is lower than in primary infections, whereas levels of IgG antibodies rise rapidly in secondary infections, even during the acute phase, thus the presence of high titers of IgG early in the course of the disease is a criterion for secondary infection. Cells of the monocyte-macrophage lineage are the major sites of viral replication. They target vascular

endothelium, platelets and various organs leading to vasculopathy and coagulopathy responsible for the development of haemorrhage and shock.<sup>5</sup> Virus antibody complexes have been detected on platelet surface of DHF patients suggesting a role for immune mediated destruction of platelet. The release of high levels of platelet activating factor by monocytes may induce platelet consumption and augment adhesiveness of vascular endothelial cells resulting in thrombocytopenia.<sup>4</sup> The presence of IgM antibodies in the sera of DHF cases cross react with platelets. These autoantibodies are involved in pathogenesis of dengue.<sup>6</sup> Leucopenia in dengue fever may be caused by virus-induced destruction or inhibition of myeloid progenitor cells.<sup>7</sup> There is an increase in aminotransferases, mainly AST has been associated with disease severity and serves as an early indicator of dengue infection.<sup>8</sup> AST and ALT were found to be increased 5-10 times in dengue fever due to liver parenchymal damage caused by the virus.<sup>9,10</sup> Criteria for grading of dengue-DF/DHF/DSS. DF-Fever of 2-7 days with two or more of following: headache, retro-orbital pain, myalgia, arthralgia with or without leukopenia, thrombocytopenia and no evidence of plasma leakage. DHFI-above criteria plus positive tourniquet test and evidence of plasma leakage. Thrombocytopenia with platelet count less than 100000/cumm and HCT rise more than 20 % over baseline. DHFII- above criteria plus some evidence of spontaneous bleeding in skin or other organs (Black tarry stool, epistaxis, gum bleeds) and abdominal pain. Thrombocytopenia with platelet count less than 100000/cumm and HCT rise more than 20 % over baseline. DHFIII (DSS)-above criteria plus circulatory failure (weak pulse, narrow pulse pressure <20mmHg, Hypotension, cold clammy skin, restlessness). Thrombocytopenia with platelet count less than 100000/cumm and HCT rise more than 20% over baseline. DHFIV (DSS)-Profound shock with undetectable blood pressure or pulse. Thrombocytopenia with platelet count less than 100000/cumm and HCT rise more than 20% over baseline. Expanded dengue syndrome (EDS)-Mild or Severe organ involvement may be found in DF/DHF, the illness commonly begins abruptly with high fever accompanied by facial flushing and headache. Anorexia, vomiting, epigastric discomfort, tenderness at right costal margin and generalized abdominal pain are common.<sup>4</sup> A positive tourniquet test is the most common hemorrhagic phenomenon. Lab diagnosis of dengue.

**Immunological response based tests:** IgM and IgG antibody assays. IgM antibodies are detectable by days 3-5 after the onset of illness, rise quickly by about two weeks and decline to undetectable levels after 2-3 months. IgG antibodies are detectable at low level by the end of the first week, increase subsequently and remain for a longer period. Because of the late appearance of IgM antibody i.e. after five days of onset of fever, serological tests based on this antibody done during the first five days of clinical illness are usually negative. During the secondary dengue infection (when the host

has previously been infected by dengue virus), antibody titres rise rapidly. IgG antibodies are detectable at high levels, even in the initial phase and persist from several months to a lifelong period. IgM antibody levels are significantly lower in secondary infection cases.<sup>11</sup>

**Isolation of Dengue Virus:** Specimens include serum, plasma or washed buffy coat from the patient, autopsy tissues from fatal cases, especially liver, spleen, lymph nodes and thymus and mosquitoes collected in nature. Isolation of the virus takes 7-10 days, hence it may not be very useful for starting the management of patients with DF/DHF.<sup>4</sup>

**Serological tests:** Haemagglutination-Inhibition (HI), Complement Fixation (CF), Neutralization test (NT), IgM capture ELISA (MAC-ELISA) and Indirect IgG ELISA. MAC-ELISA has become widely used test in the past few years. It is a simple, rapid test. It is based on detection of the dengue-specific IgM antibodies in the test serum. It has become an invaluable tool for surveillance of Dengue. It is especially useful for hospitalized patients who are generally admitted late in the illness after detectable IgM is already present in the blood.

**Haematological tests:** The white blood cell (WBC) count may be normal or with predominant neutrophils in the early febrile phase. Initial leucopenia and leukocyte count returning to normal by ninth to tenth day after therapy in most of the cases. The platelet counts are normal during the early febrile phase. According to WHO-2009, thrombocytopenia is seen on 3 to 7 day. The haematocrit is normal in the early febrile phase. A slight increase may be due to high fever, anorexia and vomiting. A sudden rise in haematocrit is observed simultaneously or shortly after the drop in platelet count. Haemoconcentration or rising haematocrit by 20% from the baseline, e.g. from haematocrit of 35% to  $\geq 42\%$  is objective evidence of leakage of plasma. A rise in haematocrit occurs in all DHF cases, particularly in shock cases. Haemoconcentration with haematocrit increases by 20% or more is objective evidence of plasma leakage. Other common findings are hypoproteinemia/albuminaemia (as a consequence of plasma leakage), hyponatremia and mildly elevated AST ( $\leq 200$ U/L) with the ratio of AST:ALT  $>2$ . In most cases, assays of coagulation and fibrinolytic factors show reductions in fibrinogen, prothrombin, factor VIII, factor XII and antithrombin III.

**Detection of antigens:** The NS1 gene product is a glycoprotein produced by all flaviviruses and is essential for replication and viability of the virus. NS1 antigen appears as early as Day1 after the onset of the fever and declines to undetectable levels by 5-6 days. Hence, tests based on this antigen can be used for early diagnosis. ELISA and dot blot assays directed against the envelop/membrane (EM) antigens and nonstructural protein 1 (NS1) demonstrated that this antigen is present in high concentrations in the sera of the dengue virus-infected patients during the early clinical phase of the

disease and can be detected in both patients with primary and secondary dengue infections for up to six days after the onset of the illness.<sup>11</sup>

**Viral nucleic acid detection:** Dengue viral genome which consists of RNA can be detected by reverse transcriptase polymerase chain reaction (RT-PCR) assay and real time RT-PCR. They offer better specificity and sensitivity compare to virus isolation with a much more rapid turnaround time.

**Management of dengue:** A full blood count of the patient should be done at the first visit. A rapidly decreasing platelet count in parallel with a rising haematocrit compared to the baseline is suggestive of progress to the plasma leakage/critical phase of the disease.<sup>12</sup> Management of dengue fever is symptomatic and supportive. Bed rest is advisable during the acute phase. Antipyretics may be used to lower the body temperature. Aspirin/NSAIDs like Ibuprofen, etc should be avoided since it may cause gastritis, vomiting, acidosis, platelet dysfunction and severe bleeding. Paracetamol is preferable. Oral fluid and electrolyte therapy is recommended for patients with excessive sweating or vomiting. Intravenous fluid should be administered if the patient is vomiting persistently or refusing to feed. Haematocrit should be determined daily especially from the third day until the temperature remains normal for one or two days. DHF I and II- Any person who has dengue fever with thrombocytopenia, high haemoconcentration and presents with abdominal pain, black tarry stools, epistaxis, bleeding from the gums etc. needs to be hospitalized. Prophylactic platelet can be given if platelet count decreased below  $<10,000$ /cmm. Dengue patients should preferably receive single donor apheresis platelets (SDAP) as compared to random donor platelets (RDP) to lower the risk of alloimmunization.<sup>13</sup> Complication can occur like metabolic acidosis and severe bleeding due to DIC and multi-organ failure. Metabolic abnormalities are frequently found as hypoglycemia, hyponatremia, hypocalcemia and occasionally, hyperglycemia.

## METHODS

The present study was conducted in the Department of Pathology, affiliated with a Tertiary care hospital. It includes 82 patients with dengue fever admitted in medicine wards from August 2015 to August 2016. All patients were suffering from dengue, confirmed by ELISA-IgM/NS1/PCR test. Haematological profile, biochemical profile and clinical signs and symptoms from the day of admission are collected. Samples for hematological data were analyzed using micros 60, three-part fully automated haematology analyzer. Fully automated clinical chemistry analyser-erba XL-640 was used for the biochemistry. Tourniquet test: The tourniquet test (TT) is a physical examination technique that can identify and stratify dengue disease. The resulting petechiae (cutaneous pinpoint, non-raised, purplish-red

spots) can be found in patients with and DHF. The TT is performed by inflating a blood pressure cuff midway between the systolic and diastolic blood pressure on a person's upper arm. After five minutes, if the number of petechiae counted in an area exceeds a certain number (20 petechiae in a one square inch area), then the test result is considered positive. Tourniquet test was carried out in all patients on admission.

All cases were graded according to severity criteria based on the technical guidelines from the WHO: DF/DHFI/DHFII/DHFIII/DHFIV. Grade III and IV are collectively called as Dengue Shock Syndrome. Data of complete blood count like haemoglobin, HCT, platelet and WBC count were collected. These data were collected on 1<sup>st</sup>, 3<sup>rd</sup> and 7<sup>th</sup> days of the admission and values were compared between different days. Normal reference ranges of parameters are: Platelet: 1.5-4.0lac/cmm, WBC: 4000-11000/cmm, HCT: 40-54%, Hb:13.5-17gm %, WBC count <4000/cmm is considered leucopenia, platelet count <150000/cmm was considered thrombocytopenia. Level of transaminase-Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were noted. AST and ALT >45 U/L were considered elevated. Clinical symptoms and signs like fever, headache, myalgia, arthralgia, rash, vomiting, diarrhea, altered consciousness, breathlessness, ascites, hepatomegaly, pleural effusion, splenomegaly, petechial and sub-conjunctival haemorrhage were recorded.

**RESULTS**

In the present study, total 82 Dengue positive cases were studied, 52 (63%) were males and 30 (37%) were females (Table 2). The male: female ratio was 1.7:1. Out of 82 patients, majority i.e. 31 (37.8 %) were in age group of 15 to 25 years followed by 29 (35.38%) in the age group of 26 to 35 years (Table 1).

**Table 1: Distribution of cases according to age.**

Age (years)	Cases (no.)	Cases (%)
15-25	31	37.8 %
26-35	29	35.38 %
36-45	13	15.82 %
46-55	5	6.11 %
56-65	3	3.67 %
66-75	1	1.22 %
Total	82	100 %

**Table 2: Distribution of cases according to sex.**

Gender	Cases (no.)	Cases (%)
Male	52	63.4 %
Female	30	36.6 %
Total	82	100 %

In present study, 24 (29%) were observed in September 22 (27%) observed in October 19 (23%) observed in

August (Table 3) No patients were observed in month of March, April, May and June. All the 82 patients presented with fever 82 (100%). Out of 82 patients, 12 (14.60%) had positive tourniquet test, 30 (36.60%) had pleural effusion, 26 (31.70%) had ascities, 11 (13.40%) had petechial spots, 24 (29.30%) had hepatomegaly, 8 (9.80%) had splenomegaly and 3 (3.60%) had sub-conjunctival haemorrhage (Table 4).

**Table 3: Distribution of cases according to calendar month (Season).**

Month	Cases (no.)	Cases (%)
August	19	23.15 %
September	24	29.27 %
October	22	26.83 %
November	8	9.77 %
December	3	3.66 %
January	2	2.44 %
February	1	1.22 %
July	3	3.66 %
Total	82	100 %

**Table 4: Distribution of cases according to symptoms.**

Symptoms	Cases (no.)	Cases (%)
Fever	82	100 %
Arthralgia	72	87 %
Myalgia	49	59 %
Headache	61	74.4 %
Abdominal pain	29	35.4 %
Rash	13	15.6 %
Vomiting	15	18.3 %
Diarrhoea	11	13.4 %
Melena	8	9.6 %
Altered sensorium	4	4.8 %
Breathlessness	3	3.6 %

**Table 5: Distribution of patients according to platelet count.**

Platelet count (Range)	Cases (no.)	Cases (%)
20000-39000/ul	17	20.7 %
40000-79000/ul	35	42.68 %
80000-99000/ul	19	23.1 %
>100000/ul	11	13.4 %
Total	82	100 %

**Table 6: Distribution of patients according to WBC count.**

Range of WBC count	Cases (no.)	Cases (%)
1100-2000	6	7.3 %
2100-3000	26	31.7 %
3100-4000	21	25.6 %
>4000	29	35.3 %

In present study out of 82 patients, thrombocytopenia was present in 86.5% of patients and majority i.e. 35 (42.68%) had platelet count between 40000-79000/cmm (Table 5). Mean value of Hemoglobin on 1st day was 12.8% and it was decreased on 3rd and 7th day respectively (12.5% and 12.2%). Majority of the patients have WBC count more than 4000/cmm (Table 6). Majority cases were of classical dengue fever 51 (62.20%), 14 (17.07%) were of DHF I, 12 (14.63%) were of DHF II, 3 (3.66%) were of DHF III and only 2 (2.44%) were of DHF IV. Out of 82, 2 patients of DHF IV died due to ARDS and shock. One patient had 60% HCT, 2800/cmm WBC count, 79000/cmm platelet count and had fever, vomiting, melena, pleural effusion and headache. Other patient had 58.4 % HCT, 7600/cmm WBC count, 2300/cmm platelet count and had similar complain with rash.

## DISCUSSION

Due to changing climate, urbanization, poor living conditions and inadequate waste management, vector born diseases like dengue fever are becoming more common. Prevalence of *Aedes albopictus* and *Aedes aegypti* together with circulation of dengue virus of more than one type in any particular area tends to be associated with outbreaks of DHF/DSS.<sup>12</sup> As most of the patients suffering from DF or DHF needs only supportive treatment therefore, it is important to have strong clinical suspicion before initiating treatment. The below-mentioned signs and symptoms when combined with the triad of raised hematocrit, thrombocytopenia and elevated liver enzymes are the main indicators of DF or DHF. Classic DF is usually a self limiting-febrile illness, whereas, DHF can cause a life threatening disease. Without proper treatment, DHF case fatality rates can exceed 20% whereas with supportive therapy; it can be reduced to less than 1%.<sup>14</sup>

In the present study, there was a male predominance with about 63.4% of patients being male and 36.6% female. Sharma S et al, Rashmi et al, Agarwal et al also found the similar findings.<sup>15-17</sup> This is due to the Asian culture whereby males spend more time outside their houses and are more likely to be exposed to mosquitoes compared to females. In present study, majority of patients were observed in month of September, October and November. Trupti et al, also found the similar findings.<sup>18</sup> In present study, all patients had fever (100%) and rash was observed in 15.6% cases. In present study, 12 (14.6%) patient had positive tourniquet test, while in Raman et al, it was 6%, Rachel et al study, it was in 33.6% of patients.<sup>19,20</sup> In present study, petechia was observed in 11 (13.4%) patient, while in Regina et al study it was 13.4% and in Raman et al it was in 43% of patients.<sup>19,21</sup>

In present study, hepatomegaly was observed in 24 (29.3%) patients whereas in Raman et al study and Regina et al study it was 10.5% and 10.4% respectively.<sup>19,21</sup> In present study, thrombocytopenia was

observed in 86.5% of cases which is supported by Priyanka et al and Avarebeel et al, who also observed 59% and 99%.<sup>22,23</sup> In present study, mean value of platelet count on 1<sup>st</sup> day was (87207.31), mean value of platelet count decreased on 3<sup>rd</sup> day (74902.434) and again on 7<sup>th</sup> day mean value of platelet count was increased (111585.37). There was a significant difference between platelet counts of day1, day 3 and day 7 according to mean and standard deviation ( $p$  value<0.05). It is supported by comprehensive guidelines for control and prevention of DF and DHF (revised and expanded edition WHO 2011) which states that thrombocytopenia is observed between day three and ten.<sup>11</sup>

In present study, leucopenia was observed in 64.3% patients. Priyanka et al, and Avarebeel et al, observed leucopenia in 44% and 41.04% respectively.<sup>22,23</sup> Leucopenia is observed when infection is caused by more virulent strain. In present study, mean value of WBC count on 1<sup>st</sup> day was (5021.95). It decreased on 3<sup>rd</sup> day (4260.976) and again increased on 7<sup>th</sup> day (4312.20) There was a significant difference between WBC counts of 1<sup>st</sup> and 3<sup>rd</sup> day and 1<sup>st</sup> and 7<sup>th</sup> day according to mean and standard deviation ( $p$ <0.05).

It is supported by Gajera et al, study who also observed initial leucopenia and then leukocyte count returning to normal by ninth to tenth day after therapy in most of the cases.<sup>24</sup> It indicates that leukocyte count is an important benchmark for clinical improvement. It is also supported by S. B. Halsted study who observed decrease in WBC count from 3<sup>rd</sup> day and increase in it by 8<sup>th</sup> and 9<sup>th</sup> day.<sup>2</sup> In present study there was no significant differences between HCT of 1<sup>st</sup>, 3<sup>rd</sup> and 7<sup>th</sup> day according to mean and standard deviation ( $P$  >0.05). The reason for this is because hemoconcentration usually occurs in patients with dengue shock syndrome and was not much altered in classical dengue fever. Rusmavati et al, study who also observed similar findings.<sup>25</sup>

In present study increased AST level was observed in 79.3% patients, Rachel et al study also observed increased AST level in 83.9% patients, Nazish Butt et al observed it in 100% patients.<sup>20,14</sup> In present study, increased ALT level was observed in 42.7% patients. Priyanka et al, study also observed increased ALT level in 40% patients, whereas Raman et al observed it in 92% patients.<sup>19,22</sup> In present study, majority patients had classical dengue fever 62.20%, followed by DHF I (17 %), DHF II (14.6 %), DHF III (3.6 %). It is supported by study conducted by Raman et al who also observed classical dengue fever 56%, DHF I 13 %, DHF II 14.6 % and DHF III 1.5%.<sup>19</sup> In present study, cases of DHF IV is 2.4 %, whereas no cases were observed in Raman et al study.<sup>19</sup>

In present study, mortality rate was 2.44%. Jain A et al, study who also observed mortality rate of 1.7% whereas it was higher in Ahmed F et al study that is 6.4 %.<sup>26,27</sup> Mortality in dengue infection is mostly due to hemorrhagic manifestation, fluid leakage and DSS.

Shock, multifocal bleeding and neurological manifestation are poor prognostic manifestations. Highly trained staff and enough resources can reduce the mortality even further.<sup>27</sup>

## CONCLUSION

Dengue is a self-limiting disease, so by observing above parameters, prognosis of dengue can be known. Peak of disease is in late monsoon and post monsoon season. It reflects the seasonal trend of dengue and so it can also help in vector control programs. The results of present study have highlighted the importance of clinical examination, haematological and biochemical profile in dengue patients. Variation in hematocrit, leucopenia, thrombocytopenia, raised liver enzymes and presence of various clinical features like fever, headache, rash, pleural effusion and other gave enough clues so as to diagnose dengue cases in their initial stages and thus facilitate early treatment and observation of dengue cases. This would minimize morbidity and mortality arising out of serious complications of DF.

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