

## Predictors of Severity of Dengue Fever in Tertiary Care Hospitals

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### ABSTRACT

**BACKGROUND:** Dengue fever, an emerging public health issue in Pakistan bears considerable morbidity and mortality. This descriptive cross sectional study was conducted to analyze clinical, hematological and serological characteristics of dengue fever variants and to identify biomarkers that predict its severity.

**METHODS:** 105 dengue cases (>12 years) were selected after ethical approval from Rawal Institute of Health Sciences & Benazir Bhutto Hospital Rawalpindi over 6 months (July to Dec 2015). Patients having pre-existing hematological disorder, liver disease, malaria and typhoid co-infection were excluded. Demographic data, clinical findings, hematological and serological profile documented. Patients were classified as classic dengue fever (DF), dengue hemorrhagic (DHF) and dengue shock syndrome (DSS). Data analyzed via SPSS version 17.

**RESULTS:** Among 105 cases, there were 79(75%) males and 26(25%) females. Mean age was 30  $\pm$ 12.8 years and mean duration of symptoms 5  $\pm$ 2 days. Dengue fever was found in 75(75%), dengue hemorrhagic fever 24(23%) and dengue shock syndrome 2(2%). Gender, mean age and duration of symptoms were comparable between DF, DHF and DSS. Common clinical features were fever (100%), headache (56%), muscle pain (43%), vomiting (43%), retro-orbital pain (23%), bleeding (12%) and hypotension (10%). Thrombocytopenia, leukopenia and pancytopenia were frequent in DHF vs. DF. Dengue NS-1 antigen positive in 71(90%) of DF cases vs 16(57%) DHF and 1(50%) DSS. Dengue-IgM positive in 32(47%) DF vs. 19(79%) DHF and 2(100%) DSS. Dengue -IgG detected in 33(42%) DF vs. 17(71%) DHF and 1(50%) DSS. 101(96%) dengue cases were treated successfully and one case expired.

**CONCLUSION:** Dengue-IgG and IgM are better predictive variables for dengue hemorrhagic fever as compared to NS-1 antigen that predicts classic dengue fever. Utilizing these predictive variables, imminent severe dengue may be identified and with vigilant monitoring, fluid resuscitation and pre-hand arrangement of blood products we may reduce complications and mortality in high risk cases.

**KEY WORDS:** Dengue fever. Dengue hemorrhagic fever. Dengue shock syndrome.

*This article may be cited as:* Shams N, Amjad S, Yousaf N, Ahmed W, Seetlani NK, Qaisar N, Samina. Predictors of Severity of Dengue Fever in Tertiary Care Hospitals. J Liaquat Uni Med Health Sci. 2016;15(04):168-73. doi: 10.22442/jlumhs.161540487

### INTRODUCTION

Dengue fever is an arthropod borne disease caused by Dengue virus having four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). It is transmitted to humans by bite of an infected female mosquito (*Aedes Aegypti*). History of Dengue date back to 1846 when epidemic was recorded in Brazil. As per estimates of WHO, 2.5 million people reside in high risk area for Dengue with approx. 50 million cases reported per year.<sup>1</sup> The prevalence of dengue shows seasonal variation with significant rise during rainfall and elevated temperature.<sup>2</sup> In 1982, twelve cases of dengue were detected

in Pakistan and the first epidemic of dengue was observed in 1994.<sup>3,4</sup> The subsequent outbreaks showed that dengue fever epidemic is at rise in Pakistan.<sup>5-7</sup>

The incubation period of dengue fever is 3-15 days (5-8 days in maximum cases). Dengue has wide spectrum of presentations including undifferentiated fever (viral syndrome), classic dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).<sup>8</sup> The diagnostic tests of dengue include dengue virus non-structural antigen non-structural protein (NS-1) by ELIZA during early phase of infection. This is followed by detectable dengue-IgM (5-6

days) and then dengue-IgG antibodies (2 weeks). The combination of NS-1 and dengue IgM is recommended for diagnosis during early phase. However, during the late incubation period and early phase of infection the above mentioned tests are negative and viral isolation in cell culture, immunofluorescence or immunohistochemistry have to be performed.<sup>9</sup>

There is no specific anti-viral therapy for dengue and mainstay of therapy is supportive and symptomatic with intravenous fluids and electrolytes management. Those with severe thrombocytopenia or active bleed may require transfusion of blood products. Timely diagnosis and intervention with close monitoring can reduce the mortality to <1%.<sup>10</sup>

Pakistan is facing frequent epidemics of dengue fever since last decade. In a Karachi based study, Wasay M et al concluded that there are changes in regional pattern and outcome of dengue over a period of years.<sup>11</sup> We conducted this study during recent dengue outbreak to document the clinical, hematological and serological characteristics of dengue fever variants and to identify biomarkers that predict its severity.

## SUBJECTS AND METHODS

This descriptive cross sectional study was conducted at Dept. of Medicine, Dengue units at Rawal Institute of Health Sciences & Benazir Bhutto Hospital (BBH) Rawalpindi (July to Dec 2015) after ethical approval and informed consent. Consecutive convenience sampling done and all indoor cases of dengue fever (age  $\geq 12$  years) meeting inclusion criteria were selected. Those with other causes of thrombocytopenia i.e. pre-existing hematological disorder, idiopathic thrombocytopenic purpura, liver disease, co-infection with malaria or typhoid fever were excluded.

Demographic details, presenting complaints and systemic examination findings were documented. Their blood complete picture, dengue NS-1 antigen, dengue -IgG and IgM antibodies performed. The duration of hospital stay, management plan and final outcome documented. Data was entered and analyzed by SPSS version 17. Frequencies and percentages calculated for qualitative variables. Mean and standard deviation calculated for quantitative variables. Chi-square test applied as a test of significance to study

association of stages of dengue with qualitative variables; and student t-test to study association with quantitative variables.

## RESULTS

Among 105 cases, there were 79(75%) males and 26 (25%) females. Mean age was  $30 \pm 12.8(13-80)$  years and mean duration of symptoms  $5 \pm 2(2-11)$  days. 31% cases were from lower socioeconomic class, 64% from middle and 5% from upper. Hospital visit was suggested by family in 45%, self 35%, friends 2% and local practitioner 7% (**Table I**).

As per diagnosis; 79(75%) had DF, 24(23%) DHF and 2(2%) DSS. 76% cases were managed in isolation wards and 24% in critical care unit. Frequent presenting features were fever in 100%, headache 56%, muscle pains 43%, nausea and vomiting 43%, retro-orbital pain 23%, bleeding 12% and hypotension 10% (**Figure I**).

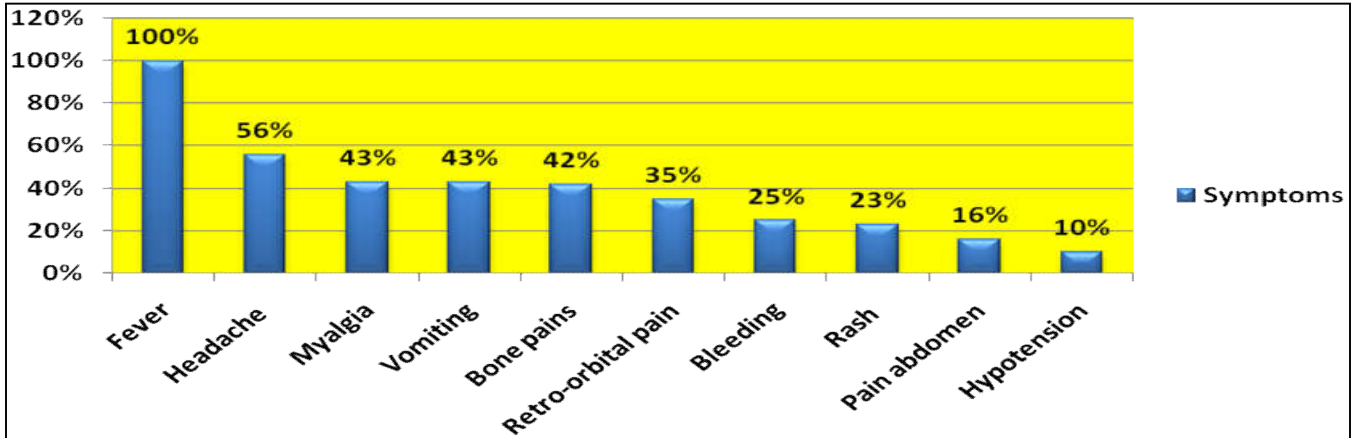
Mean hemoglobin was  $13.8 \pm 2.17 (8.4-18.6)$  g/dl. White cell counts  $4.53 \pm 2.43 (1.4-14.7) \times 10^9/L$  and platelets  $108,904 \pm 5,7462 (14,000-364,000)$ . Anemia was present in 6(25%) DHF vs. 16(20%) DF. Leukopenia in 18(75%) DHF vs. 32(41%) DF and 1(50%) DSS. Thrombocytopenia in 23(96%) DHF vs. 57(72%) DF and 2(100%) DSS. Pancytopenia in 7(29%) DHF vs. 5(6%) DF. Platelets were  $\leq 100,000$  in 79% DHF vs. 43% DF.

As per serology, NS-1 antigen was positive in 88 (84%), dengue-IgM in 48(46%), dengue-IgG in 53 (50%), both dengue IgG and IgM in 20(19%), and all of these (NS1,IgG and IgM) in 26(25%) dengue cases. Dengue NS-1 antigen was positive in 71(90%) DF cases vs. 16(57%) DHF and 1(50%) DSS; dengue-IgM in 32(41%) DF vs. 19(79%) DHF and 2(100%) DSS; dengue-IgG in 33(42%) DF vs. 17(71%) DHF and 1(50%) DSS; both IgG and IgM in 12(50%) DHF vs. 15(16.5%) DF (**Table I**).

Intravenous fluids were administered in 41(39%) cases, platelet concentrates in 7(6%) and red cell products in 2(2%). Rare complications were seen in 2 cases including pericardial and pleural effusion. 101 (96%) patients were successfully managed and discharged, 3 left against medical advice, 1 case expired and none was referred.

**TABLE I: THE DEMOGRAPHIC, SEROLOGICAL AND HEMATOLOGICAL CHARACTERISTICS IN VIEW OF VARIOUS STAGES OF DENGUE FEVER**

Variables		Dengue Fever (DF) n=79	Dengue hemorrhagic (DH) n=24	Dengue Shock (DSS) n=2	P-value
<b>Gender</b>					
Males	79(75%)	60(76%)	17(71%)	2(100%)	0.628**
Females	26(25%)	19(24%)	7(29%)	0(0%)	
Age (mean $\pm$ SD)	30 $\pm$ 12.8	30 $\pm$ 12	29 $\pm$ 14	17 $\pm$ 1	0.882**
Duration of symptoms	5 $\pm$ 2	5.1 $\pm$ 2	5.4 $\pm$ 2	4 + 1	0.523**
Duration of admission	5 $\pm$ 2.6	4.4 + 1.8	6.7 + 3.9	8.5 $\pm$ 2	<0.0001**
<b>Symptoms</b>					
Fever	105(100%)	79(100%)	24(100%)	2(100%)	<0.0001*
Rash	24(23%)	14(18%)	10(42%)	0(0%)	0.037*
Myalgia	45(43%)	33(42%)	11(46%)	1(50%)	0.920*
Headache	59(56%)	43(54%)	15(62.5%)	1(50%)	0.772*
Hemorrhage	26(24.7%)	0(0%)	24(100%)	2(100%)	<0.0001*
Pain abdomen	17(16%)	12(15%)	5(21%)	0(0%)	0.662*
Vomiting	45(43%)	35(44%)	8(33%)	2(100%)	0.163*
Hypotension	11(10%)	4(5%)	5(21%)	2(100%)	<0.0001*
Bone pain	44(42%)	32(40.5%)	11(46%)	1(50%)	0.874*
Eye pain	37(35%)	31(39%)	6(25%)	0(0%)	0.253*
Others (pericardial effusion)	2(1.9%)	0(0%)	1(4%)	1(50%)	<0.0001*
<b>Serology</b>					
Dengue NS1	88(84%)	71(90%)	16(57%)	1(50%)	0.011*
Dengue IgM	48(46%)	32(41%)	19(79%)	2(100%)	0.001*
Dengue IgG	53(50%)	33(42%)	17(71%)	1(50%)	0.044*
Both IgM & IgG	20(19%)	13(16.5%)	12(50%)	1(50%)	0.003*
NS1, IgM & IgG	26(25%)	19(24%)	6(25%)	1(50%)	0.703*
NS1 & IgM	12(11%)	7(9%)	5(21%)	0(0%)	0.238*
<b>Hematological</b>					
Anemia	22(21%)	16(20%)	6(25%)	0(0%)	0.673*
Leukopaenia	51(49%)	32(41%)	18(75%)	1(50%)	0.046*
Thrombocytopaenia	82(78%)	57(72%)	23(96%)	2(100%)	0.037*
Bicytopaenia	48(46%)	35(44%)	12(50%)	1(50%)	0.880*
Pancytopaenia	12(11%)	5(6%)	7(29%)	0(0%)	0.008*
<b>Range of platelets</b>					
$\leq$ 50,000	11(10%)	3(4%)	7(29%)	1(50%)	0.002*
51,000-100,000	44(42%)	31(39%)	12(50%)	1(50%)	
101,000-149,000	29(28%)	25(32%)	4(17%)	0(0%)	
$\geq$ 150,000	21(20%)	20(25%)	1(4%)	0(0%)	
<b>Admission area</b>					
Isolation ward	26(25%)	75(5%)	3(12%)	0(0%)	<0.0001*
Critical care unit	79(75%)	4(5%)	21(88%)	2(100%)	
<b>Outcome</b>					
Discharged	101(96%)	76(96%)	23(96%)	2(100%)	0.359*
Expired	1(1%)	0(0%)	1(4%)	0(0%)	
LAMA	3(3%)	3(4%)	0(0%)	0(0%)	
<i>(Test of significance * Chi-square; **t-test; Significant values in bold text)</i>					

**FIGURE I: BAR GRAPH PRESENTATION OF SYMPTOMS OBSERVED IN DENGUE CASES (n=105)**

## DISCUSSION

Dengue fever is a mosquito borne disease with a spectrum of clinical presentations and outcome. During the last decade, this pandemic has been witnessed in various countries all over the world. In Pakistan, dengue has been endemic for decades in the southern port city of Karachi, but large epidemics in the north-east emerged since 2011 in Lahore.<sup>12</sup> In 2013, the second large epidemic occurred in north-eastern Pakistan in Punjab and Khyber-Pakhtunkhwa (KP) provinces, establishing the region as an emerging focus of seasonal dengue epidemics.<sup>13</sup>

Amongst 105 patients inducted in current study, 75% were males and 25% females. Other studies<sup>14,15</sup> had also reported male preponderance for dengue fever, reason may be increased outdoor working habit of men as compared to women.

In current study, the mean age of presentation was  $30 \pm 12.8$  years. In South-east Asia the average age of patients with dengue infection is increasing over years. In Thailand, affected adults > 15 years of age comprise 30-40% of dengue cases.<sup>16</sup>

As per dengue stage, 75% patients had classic DF, 23% had DHF and 2% had DSS. Similar results have been observed in several previous studies showing that majority of the patients have simple dengue fever and fewer develop complicated infection that is likely to be secondary to variation in immune response after exposure to virus. However data from regional studies suggests that over a period of years there is change in trend of variants and more cases of dengue hemorrhagic fever observed in subsequent epidemics.<sup>11</sup>

The majority of the patients presented with fever 100%, headache 56%, muscle pains 43% and vomiting 43%. Retro-orbital pain was found in 23%, abdominal pain 16%, rash 15%, bleeding 12% and hypotension 10% (**Fig I**). Kumar et al<sup>17</sup>, reported almost identical presentation, however in contrast to current

study they do not found retro-orbital pain as the presenting symptom. Another study by Babalichi et al shows similar pattern of presenting symptoms.<sup>18</sup> Most of the symptoms and their duration didn't differ in patients with DF and those with DHF and DSS in current study except hypotension, hemorrhage and rash ( $p < 0.05$ ).

Majority of the patients had leucopenia (49%) and thrombocytopenia (78%) on complete blood picture. Anemia was found in 21%, bicytopenia in 46% and pancytopenia in 11%. Leukopenia was significantly more in patients with DHF than DF (75% vs.41%;  $p = 0.046$ ). Carrsaco et al<sup>19</sup> found no association of leucopenia with severity of dengue fever, in contrast during present study we found statistically significant leucopenia in patients with DHF than DF (75% vs.41%;  $p = 0.046$ ).

Pancytopenia was also significantly more in patients with DHF (29%) than classic DF (6%) in current study. This may be related to severe immune mediated bone marrow suppression in patients with DHF.

For current study, In majority of the patients, platelet counts remained above 50,000 and in a small number (i.e. 20%) the counts dropped below 50,000. However a study conducted in Brazil<sup>20</sup> had contrary results; platelet count of  $\leq 50,000$  platelets/mm<sup>3</sup> was found in 70% DF cases, 29% DHF and 50% DSS. Hence, lower platelet counts at admission may be associated with severity of dengue spectrum as shown in some studies.<sup>21,22</sup>

Dengue serology of the patients in our study showed that 84% had positive NS-1 antigen, 46% were dengue-IgM positive and 50% dengue-IgG positive (Table I). NS-1 was positive in 90% of patients with simple DF as compared to 57% of patients with DHF. Both IgM and IgG were found in 50% of the patients with DHF and DSS while only 16.5 % of patients with DF had both markers positive. These results show increased association of complicated DF (i.e. DHF) with



possible previous sensitization to dengue virus resulting in augmented immune response and increased incidence of plasma leakage. When dengue-IgM antibodies are produced, various cytokines are released that results in increased vascular permeability and coagulopathy. In patients with secondary infection, there is enhanced dengue-IgG antibody binding to heterologous virus. This further leads to infection of antigen presenting cells, therefore contributing to increased viral load and activation of T cells. This further augments the release of inflammatory cytokines leading to development of severe disease.<sup>23</sup>

The results of the current study showed that leucopenia, thrombocytopenia with platelet counts less than 50,000 and presence of both dengue-IgM and IgG can be used as predictor of progression to severe dengue, and therefore can be used to triage dengue patients into those that are most and least likely to develop complications. This may allow better allocation of limited healthcare resources, alleviating pressure on the healthcare system and avoiding unnecessary cost.

Majority of the patients in current study were managed with intravenous fluids (i.e. 39%), while only few required platelets transfusion (6%) and Red cell concentrates (2%). Thus prompt fluid resuscitation remains the mainstay of treatment even in patients with complicated dengue fever. In most adult cases, timely and effective intravenous crystalloid replacement of plasma losses results in a favorable outcome. If shock persists, immediate volume replacement with crystalloids should be followed by plasma or colloid solutions.<sup>24</sup> The fact that DHF and DSS patients recover quite rapidly after appropriate fluid therapy suggests that cytokines do not cause tissue destruction; rather leads to reversible endothelial cell dysfunction as shown in many immunopathology models.<sup>21</sup> Evidence suggests that red cell transfusions should be given to patients with overt blood loss i.e. ten percent or more of blood volume and in cases of refractory shock with declining hematocrit. Kaur et al concluded that there is no proven role of prophylactic platelet transfusions; rather this may lead to development of pulmonary edema and prolonged hospitalization.<sup>25</sup>

Overall prognosis of dengue fever is good with majority of the patients discharged in stable state. However patients with severe dengue need good in-hospital care with vigilant monitoring of warning signs in high dependency units and cautious management with intravenous fluids and blood products if required.

The results of current study could lead to better interpretation of regional hematological and serological trend of dengue fever. This may be helpful to identify the high risk cases of complicated dengue on the basis of demography, hematology and serology. However, certain limitations of the study need to be men-

tioned that include lack of randomization of sample and inability to perform costly investigations like viral PCR.

## CONCLUSION

There is predominance of younger patients and males among dengue cases. Thrombocytopenia, leucopenia and pancytopenia are frequent in dengue hemorrhagic fever as compared to classic dengue fever. Dengue-IgG and IgM better predict dengue hemorrhagic fever as compared to NS-1 antigen that predicts classic dengue fever. Dengue-IgG and IgM are better predictive variables for dengue hemorrhagic fever as compared to NS-1 antigen that predicts classic dengue fever. Utilizing these predictive variables, imminent severe dengue may be identified and with vigilant monitoring, fluid resuscitation and pre-hand arrangement of blood products we may reduce complications and mortality in high risk cases. Clinical suspicion should always be mainstay of the management during the epidemic.

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