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DENGUE FEVER;
CLINICO-PATHOLOGIC CORRELATIONS AND THEIR ASSOCIATION WITH POOR OUTCOME

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ABSTRACT... One of the major health hazards that has erupted in Pakistan within the recent past years and has caused loss of life of many young people is Dengue Fever. **Objective:** Main objective was to find clinico-pathologic parameters which are essentially associated with complications and contribute to the adverse outcome. **Material & Methods:** This prospective study was conducted on 106 seropositive cases of dengue fever. Patients were taken from Abbasi Shaheed Hospital, Karachi from June 2008 to March 2009. **Results:** The most common hematological abnormalities were thrombocytopenia and leucopenia. Platelets count below 50 × 10^3/ µL was seen in (78%) cases and 49 % patients had total white cell count below 4 ×10^3 /µL. Partial thromboplastin time was significantly prolonged in (26%) cases whereas prothrombin time was normal in all patients. Liver enzymes both Aspartate Aminotransferase (AST) and Alanine Amino-transferase were significantly elevated in (60%) patients. Blood urea nitrogen (BUN) and creatinine was deranged in (23%) patients. **Conclusions:** Fever was the most common clinical presentation(86 % of the patients). Hematological parameters (low platelet count, low total leucocytes count, prolonged APTT and raised hematocrit) and biochemical parameters (raised aminotransferases, blood urea nitrogen) have strong association with the complications of dengue fever and hence are associated with the poor outcome of disease.

Key words: Dengue Fever, Thrombocytopenia, Platelet Count

INTRODUCTION

Dengue virus, a mosquito-borne human viral pathogen, has recently become a major national health problem particularly in the tropical and subtropical countries, predominantly in the urban and peri-urban areas[1]. The geographic distribution of dengue virus has greatly expanded and the number of cases has dramatically increased during the past three decades[2]. The disease is transmitted among humans by mosquito Aedes aegypti mostly in rainy season.

This may be a reason why the epidemics of dengue tend to coincide with the rainy season[3]. There are four dengue virus serotypes, called DEN-1, DEN-2, DEN-3, and DEN-4. They belong to the genus Flavivirus, family Flaviviridae (of which yellow fever virus is the type species), which contains approximately 70 viruses[4].

The flaviviruses are relatively small (40-50 nm) and spherical with a lipid envelope. After a person is bitten by an infective mosquito, the virus undergoes an incubation period of 3 to 14 days (average, 4 to 7 days), after which the person may experience acute onset of fever accompanied by a variety of nonspecific signs and symptoms. During this acute febrile period, which may be as short as 2 days or as long as 10 days, dengue viruses may circulate in the peripheral blood[5]. If other A. aegypti mosquitoes bite the ill person during this febrile viremic stage, those mosquitoes may become infected and subsequently transmit the virus to other uninfected persons, after an extrinsic incubation period of 8 to 12 days[6]. Clinically, the Dengue fever may be divided in 02 types: - Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS).

The initial temperature may rise to 102 to 105˚F, and fever may last for 2 to 7 days. The fever may drop after a few days, only to relapse 12 to 24 h later (saddleback). A relative bradycardia may be noted despite the fever. The conjunctivae may be injected, and the pharynx may be inflamed. Lymphadenopathy is common. Rash is variable but occurs in up to 50% of patients as either early

or late eruptions[7]. Towards the end of the febrile phase of illness or after the temperature falls to or below normal, petechiae may appear; these may be scattered or confluent. Intense pruritus followed by desquamation on the palms of the hands and soles of the feet may occur.

Hemorrhagic manifestations in patients with dengue fever are not uncommon and range from mild to severe. Skin hemorrhages, including petechiae and purpura, are the most common, along with gum bleeding, epistaxis, menorrhagia, and gastrointestinal (GI) bleeds. Hematuria occurs infrequently, and jaundice is rare[8].

Laboratory findings associated with dengue fever include a neutropenia followed by a lymphocytosis, often marked by atypical lymphocytes. Liver enzyme levels in the serum may be elevated; the elevation is usually mild. Thrombocytopenia is also common in dengue fever.

As with dengue fever, leucopenia is common; thrombocytopenia and hemo-concentration are constant findings in DHF and DSS. A platelet count of $\leqslant 100 \times 10^3$ /µL is usually found between the days 3 and 8 of illness[9]. Hemoconcentration, indicating plasma leakage, is almost always present in classic DHF but is more severe in patients with shock.

The primary pathophysiologic abnormality seen in DHF and DSS is an acute increase in vascular permeability that leads to leakage of plasma into the extra vascular compartment, resulting in hemoconcentration and decreased blood pressure[10]. Plasma volume studies have shown a reduction of more than 20% in severe cases.

Early diagnosis and aggressive fluid replacement therapy with good nursing care can decrease fatality rates to 1% or less. Normal saline or lactated Ringer's solution can be used in patients with mild DHF and DSS, but plasma or plasma expanders may be necessary in those with severe cases.

Haemostatic changes in DHF and DSS involve three factors: vascular changes, thrombocytopenia, and coagulation disorders[11]. Almost all DHF patients have increased vascular fragility and thrombocytopenia, and many have abnormal coagulograms, suggesting disseminated intravascular coagulation, which is also evidenced by concomitant thrombocytopenia, a prolonged partial thromboplastin time, a decreased fibrinogen level, and increased levels of fibrinogen degradation products. GI hemorrhage is found at autopsy in the majority of patients who die[12].

AIMS & OBJECTIVES

Main objective of the study was to find such hematological & biochemical parameters which are essentially associated with complications and contribute to the adverse outcome. So, that patients having deranged parameters could be identified early and managed aggressively in the Intensive Care Units (ICU) where as the patients who exhibit normal parameters can be managed conservatively in wards.

OPERATIONAL DEFINITION

POOR OUTCOME

Poor outcome is defined as ,the patients who developed complications such as bleeding manifestations, hemorrhage, shock, acute tubular necrosis (acute renal failure) or fulminant hepatic failure.

MATERIAL AND METHODS

This prospective study was conducted on 106 seropositive cases of dengue fever. Patients were taken from single tertiary care hospital (Abbasi Shaheed Hospital, Karachi from June 2008 to May 2009) so that level of the treatment and care of all the patients remain same.

INCLUSION CRITERIA

Any acute febrile illness with some of the following symptoms: myalgia, headache, retro-orbital pain, bleeding, altered sensorium, shock or low platelet count and positive IgM to dengue fever were included in the study.

EXCLUSION CRITERIA

Following patients were excluded from the study. Patients with the identified bacterial focus e.g typhoid with positive dengue IgM or IgG. Patients with only IgG

positive not IgM. Any other identified specific infections e.g. malaria with positive dengue IgM or IgG and patients with inadequate data, lab parameters e.g platelet count not done.

One hundred and six (106) cases of DF/DHF were included in the study. Patients confirmed to have DF & DHF if IgM positive on ELISA.

An informed consent was obtained and a detailed clinical history, physical examination and baseline investigations were done. A specially designed Proforma was used for data collection.

Total 9.5 milliliters of blood was drawn; 2 milliliters of blood in 3mg of EDTA anticoagulant drawn for complete blood count and 4.5 ml blood in 0.5 ml citrate anticoagulant for PT and APTT and 3 ml blood in plain bottle was collected for blood urea nitrogen, serum creatinine and serum transaminases.

Blood counts including red cell count (RCC), hemoglobin (Hb), hematocrit (Hct), platelet count, total white cell count (TWCC), differential leukocyte counts (DLC) was performed on Sysmex (KX-21 automated hematology analyzer). PT and APTT was done on KC 4 Amelung (Biotech), dengue IgM was done on micro plate reader by enzyme linked immunosorbant assay (ELISA), blood urea nitrogen, serum creatinine and serum transaminases was done on micro lab 300 of Merck diagnostics.

RESULTS AND OBSERVATIONS

Total 106 patients were confirmed as having dengue viral infection by IgM- capture Elisa and included in the study. The patients were both males and females ranging from 6 to 60 years with mean age of 29.6±11.6 years as shown in table-I.

SEASONAL DISTRIBUTION

Frequency of cases varied in different months. Most of cases were seen in the summer months of July, August and September as shown in Fig -1. Various clinical features are summarized. Fever was the most common clinical presentation found in 88 patients(86%), there was no specific pattern of fever and it ranged from 38°C to 40°C, other common clinical features were headache and myalgia seen in 69 patients (66.7%), vomiting was seen in 29 patients (25.64%) and diarrhea in 23 patients (20.51%). A maculo-papular rash was seen in 29 cases (26.5%), as shown in table-II.

Table-I.

Age roup (yrs)	No. of cases (%)
0-10	03 (2.9)
11-20	16 (15.8)
21-30	45 (43.9)
31-40	25 (23.9)
41-50	09 (8.5)
51-60	08 (7.7)
Total	106 (100)

Fig-1.

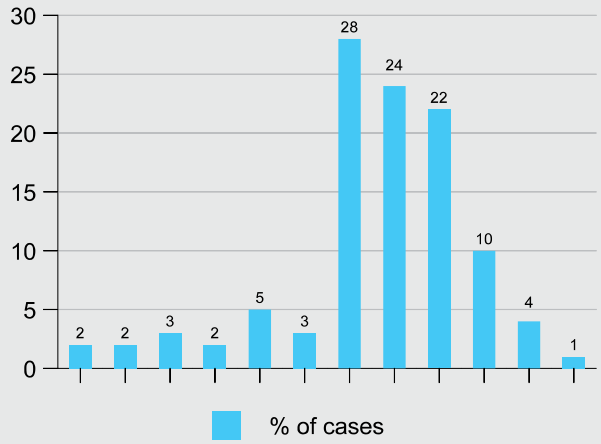


Table-II.

Symptoms	No. of cases (%)
Fever	88 (83.01%)
Headache & myalgia	69 (65.09%
Vomiting	29 (27.36%)
Diarrhea	23 (21.7%)
Maculopapular rash	29 (27.36%)

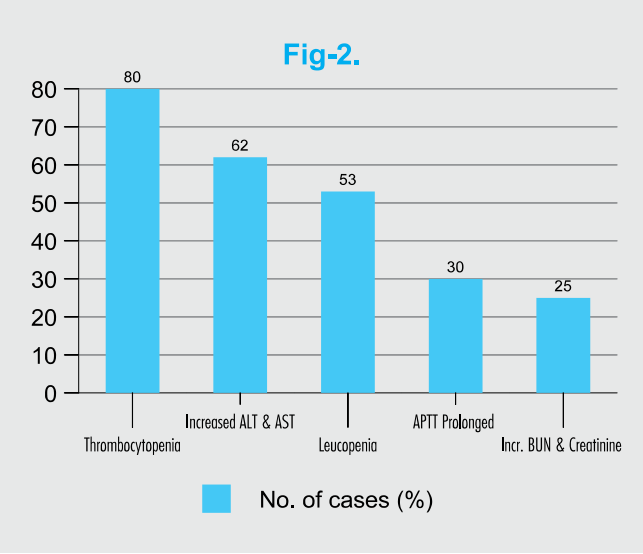
The most common hematological abnormalities were thrombocytopenia and leucopenia. Platelets count below 50 × 10^3 / µL was seen in (78%). Forty-nine percent (49%) patients had total white cell count below 4 ×10^3 /µL Partial thromboplastin time was significantly prolonged in (28%) whereas prothrombin time was normal in all patients. Liver enzymes both Aspartate Aminotransferase (AST) and Alanine Amino-transferase were significantly elevated in 60% patients. Blood urea nitrogen (Bun) and creatinine was deranged in 23% patient as shown in table-III.

Table-III.

Disturbed variables	No. of cases (%)
Thrombocytopenia	80 (78%)
Increased ALT & AST	62 (60%)
Leucopenia	53 (49%)
APTT prolonged	30 (26%)
BUN & Creatinine	25 (23%)

The mean hemoglobin and hematocrit were normal in the majority of patients. Leucopenia (<4.0 ×10^3/µL) was observed in 53 of the 106 patients for whom the total leukocyte count was available. At the time of initial presentation, 80 patients had a platelet count below 100 ×10^3/µL. In four patients, the platelet count was >100 ×10^3/µL. Severe thrombocytopenia (Platelet count <20 ×10^3/µL) was present in 43.8% of the patients. There was no correlation between the platelet counts and the presence of ecchymoses. Platelet function was studied in those six patients with hemorrhagic manifestations who had platelet count in the normal range. Of these six patients, a reduced platelet aggregation was found with adrenaline in five patients and with adenosine diphosphate (ADP) in three patients respectively. One patient showed normal platelet aggregation with adrenaline and ADP. Coagulation profile APTT was prolonged in 30 patients tested (Fig-2).

Serum bilirubin was elevated in three of the 34 patients in whom it was tested. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated in 62 of 106 patients tested.



Parameters	Complications
Thrombocytopenia (80)	Bleeding manifestations (56)
Leucopenia (53)	Complication of septicemia (35)
Raised serum transaminase level (62)	Acute liver failure (28)
Raised APTT level (30)	Bleeding manifestations (25)
Raised BUN & creatinine (25)	ATN (09)

Total no of patients who had thrombocytopenia were 80 (78%) of the total patients, out of 80 patients 56 cases developed bleeding manifestations, which were either skin manifestations like echymosis, purpura and bruises or mucocutaneous bleeding manifestations like epistaxis, hematemesis and melena. Leucopenic cases were 53(49%) and out of those 35 cases developed complications of septicemia or septic shock. Total number of 62 (60%) cases had raised serum transaminase levels and out of those, 28(26%) developed symptoms of acute liver failure. There were 30(28%) cases who had raised APTT and out of those 25(23%) of them had some sort of bleeding manifestations, mostly skin manifestations, rather mucocutaneous bleeding. Raised blood urea nitrogen and creatinine were observed in 25(23%) cases and out of those 09(14%) showed signs of acute tubular necrosis.

STATISTICAL ANALYSIS

The data were analyzed by using SPSS via Pearson chi-square. The statistical significance was considered as p-value less than the 0.05. The chi-square analysis the (p=0.22) value is greater than the 0.05 and we conclude that the parameters are associated with the complications.

Comparison of the demographic character showed that shock was more frequent in younger patients but the difference was not significant (P=0.22). Bleeding manifestations and hemorrhage were equally common in all age groups. These was no predilection for any gender among the patients who developed complications.

Among the laboratory indices, it was seen that patients who had platelet count less than 50 ×/10^3 µL developed shock more frequently (P=0.01 Or =6.5, 95%; c1=1.4 to 31.9) and those with counts above 100 ×/10^3 µL developed shock less frequently (P=0.01) odds ratio could not be estimated. Raised values of Blood urea nitrogen (BUN) and serum creatinine were found more frequently in those who developed shock (P=0.03 or=6.3,95%; C1=1 to 45). Patients who developed shock had tendency to have higher hemotocrit (because patients develop shock due to hypovolumia, which is due to hemorrhage or leakage of plasma from the endothelial cells of the blood vessels) but was not statistically significant. Bleeding was significantly related to severe thrombocytopenia (platelet counts below 50 ×/10^3 µL (P=0.01 OR=9.1, 95% C1=1.11 to 211). However bleeding were not related to moderate thrombocytopenia (Platelet count between 50 ×/10^3 µL to 100 ×/10^3 µL). Most of patients who had bleeding, had decreased hemotocrit by 20%, after treatment (P=0.01 OR=9.1, 95%; C1=1.11to 211).

The parameters found to be significantly different between these groups by univariate analysis, were then subjected to multivariate analysis to exclude the effect of other complications, as twenty five patients were found to share more than one complications.

The factors found related to shock were, low platelet count and raised parameters e.g. BUN & Creatinine. Parameters related to hemorrhagic manifestations were low platelet count and prolonged APTT. Parameter associated with ATN (acute tubular necrosis) was shock (low perfusion of kidneys) while acute hepatic failure was associated with raised transaminase levels.

DISCUSSION

Total 106 patients were included in this study with seropositive dengue IgM and frequency of distribution among the gender, age and difference in laboratory parameters was studied. Various clinical features were summarized. Fever was the most common clinical presentation (86% of the patients); there was no specific pattern of fever and peak of fever ranged from 38 C to 40C. Other common clinical features were headache (69.1%), myalgia (66.7%), vomiting (25.64%), and diarrhea (20.51%). A maculo-papular rash was seen in (20.5%). Of these patients (27%) developed Hemorrhage, 35% developed shock and 60% had bleeding manifestations.

The most common hematological abnormalities were thrombocytopenia and leucopenia. Platelet count below 50 ×10^3 /µL was seen in (78%), 49% patients had total white cell count below 4 ×10^3/µL. Partial thromboplastin time was significantly prolonged in (26%), whereas prothrombin time was normal in all patients. Liver enzymes both Aspartate Aminotransferase (AST) and Alanine Amino-transferase were significantly elevated in (60%) patients. Blood urea nitrogen (Bun) and creatinine were deranged in (23%) patients.

Similar kind of study was done in Jeddah by M Ayub and colleagues[13], which showed similar kind of derangements in laboratory parameters. It was seen that most of the cases were reported in June, July and August. Fever was most common presenting feature along with headache, vomiting and diarrhea.

In the present study it was also shown that incidence of cases of dengue fever was at its peak during July, August and September as compared to the other months of the Year.

In that study in Jeddah,[13] they proved that thrombocytopenia was most common hematological parameter noted which were seen in 58.8%, while in the present study thrombocytopenia was also most common (80%) in the patients of dengue fever.

Leucopenia, raised APTT and abnormal liver functions tests, with predominantly increase in transaminases were associated with dengue fever in that study.

The present study not only proved the association of dengue fever with these deranged laboratory parameters, but also the correlation of these parameters with the complications, hence poor outcome of disease.

In India Sunil Gomber[9] did a study on the sensitivity of the tourniquet test, in diagnosing the dengue fever. He concluded that the tourniquet test had low sensitivity and was positive in only 25% of cases. He also concluded that there was minor statistical difference in the incidence of bleeding manifestations between thrombocytopenic and non-thrombocytopenic individuals, highlighting poor association of thrombocytopenia with bleeding manifestations.In the present study it was seen that thrombocytopenia had association with the bleeding manifestation and thrombocytopenic patients not only developed mucocutaneous bleeding manifestations but also skin manifestations, like bruises, petichae and purpura.

In one study in Singapore, by Aneelies W. smith[8], "Use of Simple Laboratory Features to Diagnose the Early Stage of Dengue Fever, and to distinguish it from other viral infections, like severe acute respiratory syndrome (SARS)" it was noted that symptoms of dengue fever were maculopapular rash, some respiratory symptoms and along with non specific viral symptoms like fever, myalgia, headache and flu.

In present study fever along with headache, myalgia and rash were prominent and diarrhea, vomiting also noted but mostly with age less than 12 years. Study concluded that the laboratory features, that are highly predictive of dengue fever are, decreased platelet count, decreased white cell count and raised serum aminotransferases.

Another study was done by Maria P.Gomes[14], in Brazil, association of dengue fever and acute hepatitis .In that study it was noted that severe painful liver enlargement was followed by some non specific clinical features of dengue fever. Raised AST and ALT was seen in 46% patients.

In present study acute liver failure was also seen in the patients and followed by raised aminotransferases in (62%) patients and out of those 28 patients developed fulminant hepatic failure.

Present study, which not only showed these parameters as a diagnostic, or differentiating tool, but also suggested that these parameters could be used to distinguish the patients who are more prone to develop complications.

CONCLUSION & SUGGESTIONS

The following conclusions can be made on the basis of the above findings that the Hematological parameters (low platelet count, low total leucocytes count, prolonged APTT and raised hematocrit) and biochemical parameters (raised aminotransferases, blood urea nitrogen) have strong association with the complications (poor outcome) of dengue fever, and hence are associated with the poor outcome of disease.

Patients should be observed on these grounds and separated on the basis of laboratory parameters. The patients with deranged parameters can be separated either in intensive care units or wards specialized for dengue fever patients and require extra care so they may not develop the complications. Patients with normal hematological & biochemical parameters may be managed conservatively.

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