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# Clinical, Biochemical and Ultrasonographic Evaluation in Early Diagnosis of Dengue Haemorrhagic Fever

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## ABSTRACT

**Objectives:** The object of this study is to evaluate clinical, biochemical and ultrasonographyic findings in early diagnosis of Dengue Hemorrhagic Fever (DHF). **Design**: A case series. **Place study**: The study was carried out in Medical Department of CMH and Mayo Hospital, Lahore. **Methodology:** 351 patients suspected of DHF were enrolled in two different hospitals of Lahore during outbreak of the disease. The diagnosis was confirmed by serology. **Results**: The most common symptom in 100% patients was fever followed by musculoskeletal pain. Liver biomarkers were elevated in 237 (77.9%) patients throughout the coarse of illness, reaching peak on 3rd day and declining after 7th day of illness. The sonographic features included hepatomegaly in 234 (77.2%) patients, pleural effusion in 226 (74.6%), thickening of gallbladder wall in 221 (72.9%) and ascites in 209 (69%). **Conclusion:** Liver biomarkers and abdominal ultrasonography can be used as first line of imaging modality in patients, suspected of DHF prior to serological confirmation in dengue epidemic area.

**Key Words:** Dengue hemorrhagic fever (DHF), liver biomarkers, ultrasonography, hepatomegaly, thickening of gallbladder wall, pleural effusion.

## INTRODUCTION

Dengue is the most prevalent mosquito born viral disease. It is estimated that over 50 million dengue infection occur each year all over the world[1]. This epidemic occurs in tropical and sub-tropical areas of the world. Dengue infection was first documented in 1982 in Central Provinces of Punjab[2]. The first reported out break of dengue fever in Pakistan was in 1994[3]. Largest outbreak occurred in 2006.[4] In Punjab 21,212 cases were reported in 2010-2011 and mortality rate 1-5%. The disease is an acute mosquito transmitted viral infection disease. *Aedes aegypti* female mosquito is responsible for transmitting the disease. Out of the four virus serotype DEN-1, DEN-2, DEN-3 and DEN-4 of F1a virus cases it[5].

The disease occurs in two forms, classic diseases: the milder form of the disease and dengue hemorrhagic fever: the severe form of disease. The severity of the disease falls into 4 grades according to WHO classification[6].

**Classification of diseases severity in Dengue hemorrhagic fever**

| Severity/Grades | Clinical features |
|---|---|
| Mild | |
| Grade I | High fever and positive tourniquet test for capillary fragility. |
| Grade II | Grade 1 features plus spontaneous bleeding |
| Severe | |
| Grade III | Grades II features plus circulatory failure, with rapid, weak pulse and hypotension (systolic blood pressure <90 mmHg). |
| Grade IV | Grade III features plus profound shock, with blood pressure undetectable. |

Dengue virus may be asymptomatic or may lead to dengue fever (DF), dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). DF is an acute febrile illness taking for 2-7 days with positive tourniquet test.

The above two clinical criteria plus thrombocytopenia and haemoconcentration or rise of haematocrit are sufficient to establish the diagnosis of DHF (WHO)[6].

DSS is characterized by history of fever with haemorrhagic manifestation plus sudden onset of circulatory failure characterized by rapid pulse, hypotension, cold clammy skin and restlessness.

Two criteria *i.e.* fever with hemorrhagic manifestation like epistasis or bleeding gums or positive tourniquet with blood pressure cuff showing 20 patechial spots per square inch plus thrombocytopenia and haemoconcentration or use of haematocrit are sufficient to establish clinical disease of DHF (WHO, 1996).

Despite its clinical variability, the acute phase, begin with fever that is indistinguishable from initial phase of other febrile infection[8,9]. There is no test that can diagnose dengue infections at early stage (48-96 hrs after onset with accuracy and reliability). So diagnosis of fever is based on clinical evaluation and serology. Positive serology (anti dengue antibody) is the main stay in diagnosis of DF[10], but serology takes 5-7 days to become positive.

There is direct or indirect evidence of biochemical alterations in dengue infection. Previous studies have reported that patients with dengue hemorrhagic fever have elevated levels of serum-transaminases; aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[11], however they have not be evaluated in prospectively in early stages of dengue hemorrhagic fever[12].

Ultrasonography is rapid and non-invasive imaging technique[13,15]. Recent studies have concluded that thoracic and abdomino pelvic ultrasound can be an important adjunct to clinical profile in early diagnosis of DHF. It can be used as first line imaging modality in patients suspected of dengue infections to detect early signs of disease, prior to serological confirmation especially in dengue fever epidemic area, along with levels of serum transferase[14].

The object of this study is to evaluate clinical, biochemical markers and ultrasonographic findings in early diagnosis of DHF, which may be useful in early management of the disease, decreasing complication and mortality rate.

## PATIENTS AND METHODS

This prospective study was carried out in CMH and Mayo Hospital, Lahore. Random patients with acute onset of high grade fever less than 72 hours duration suggestive of disease were admitted as inclusion per criteria between age group 12-60 years, irrespective of gender. After conducting clinical examination, consent confirmed, blood samples were taken to determine TLC, DLC, hematocrit, platelet count, plasma ALT, AST levels. Later each patient underwent thoracic, and pelvic ultrasonography with a SSA. 340 real time ultrasound scanner equipped with 3.5 MHz curved assay tranducer with 6 hours prior fasting. On ultrasonograph emphysis was given to detection of hepatomegally, measurement of thickness of GB wall, pleural effusion and free fluid in abdomen.

### Exclusion criteria

Exclusion criteria include concomitant disease like diabetes mellitus, heart disease and auto-immune disease were excluded.

### Inclusion criteria

Patients with fever of all grades of severity, musculo-skeletal pain, rash, vomiting, between age group 12-60 years and both sex.

### Study variables

Include (a) demographic variable, age and sex (b) clinical variables: sign and symptoms and related investigation (c) sonographic variables – thickening of gallbladder wall, hepatomegally, pleural effusion, ascites and pelvic ascites.

### Serological studies

Serological test (anti-dengue antibody) was performed on 5$^{th}$ day of onset of fever to confirm the diagnosis. A case of dengue was defined as a 4-fold or greater change in reciprocal IgG antibody titre to 1 or more dengue virus antigen in serum sample or

an positive IgM antibody test on a late acute or convalescent phase by enzyme linked immunosorbent assay. Patients with negative serology were excluded from the study.

Dengue hemorrhagic patients were followed up daily for seven days of disease. For data collection a questionnaire was developed which included sign & symptoms, Lab. investigations like TLC, DLC, LFTs (bilirubin, AST, ALT, A/P), total protein, albumin, haematocrit and platelet count. Sequential analysis of liver biochemical markers as serum transferases were taken. An increase in AST level 3 times above normal value 42 U/L was considered significant. Haematocrit and platelet counts were recorded daily.

**Data analysis**

The percentages were calculated using calculation and standard statistical formulae. Data was analysed by suing SPSS version 10.

The results were expressed as mean ± S.D. $X^2$ tests was used to compare proportions $p<0.05$ was considered positive. Data was analyzed using SPSS 10.

## RESULTS

351 enrolled patients, belonged to age 12-60 years with mean age 36.7±14.59. 109 (36%) were female and 194 (64%) were male. of 28 were exempted because of co-morbid disease and 20 refused to admission. Study was carried out on 303 patients.

Sign and symptoms are tabulated in Table 1 and 2. The most common symptom is musculoskeletal pain. Hepatomegaly and positive tourniquet test were the most common signs.

**Table 1: Clinical symptoms in suspected dengue hemorrhagic patients.**

| Symptoms | No. | % | Mean±SD |
|---|---|---|---|
| Fever | 100 | 100% | 1±0 |
| Musculo-skeletal pain | 181 | 59.8% | 0.6±0.5 |
| Vomiting | 157 | 51.8% | 0.5±0.5 |
| Skin rash | 88 | 30.2% | 0.3±0.5 |
| Abdominal pain | 65 | 21.7 | 0.2±0.4 |
| Epistaxis | 41 | 13.5% | 0.1±0.3 |

**Table 2: Clinical signs in study patients.**

| Signs | No. | % | Mean±SD |
|---|---|---|---|
| Hepatomegaly | 95 | 31.5% | 0.3±0.5 |
| Rash | 83 | 27.4% | 0.3±0.5 |
| Positive tourniquet test | 64 | 20.8% | 0.2±0.4 |
| Dehydration | 62 | 20.6% | 0.2±0.4 |
| Lymphadenopathy | 41 | 13.5% | 0.1±0.3 |
| Sub conjunctival hemorrhage | 18 | 6.3% | 0.6±0.2 |

Laboratory findings in suspected dengue hemorrhagic patients are tabulated (Table 3). It was observed that 67.5% patients showed a decrease values of total leukocyte count. 53.5% and 45.7% patients showed decrease platelet count and hematocrit value respectively. 40% patients showed prolong prothrombin time and APTT. 77.97% patients showed raised liver enzymes while pleural effusion is observed in 13.7% patients.

**Table 3: Laboratory findings in suspected patients.**

| Parameters | No. | % | Mean±SD |
|---|---|---|---|
| Decreased TLC | 204 | 67.5% | 0.7±0.5 |
| DLC (Monocytosis) | 197 | 65.1% | 0.7±0.5 |
| Liver enzymes (Increase) | 237 | 77.9% | 0.5±0.5 |
| Platelet count | 162 | 53.5% | 0.5±0.5 |
| Hematocrit (Decease) | 138 | 45.7% | 0.4±0.5 |
| PT, APTT (Prolonged) | 121 | 40.0% | 0.8±0.4 |

Ultrasonographic findings in suspected dengue hemorrhagic patients is tabulated (Table 4). It was observed that hepatomegaly, pleural effusion and thickening of gall bladder wall were observed in most of the patients *i.e.* 72-77%. Ascites was observed in 69% of patients and pelvic edema was noted in 18.4% patients.

**Table 4: Ultrasonographic findings in study patients**

| Findings | No. | % | Mean±SD |
|---|---|---|---|
| Hepatomegaly | 234 | 77.2% | 0.8±0.4 |
| Pleural effusion | 226 | 74.6% | 0.8±0.4 |
| Thickening of GB wall | 221 | 72.9% | 0.7±0. 5 |
| Ascities | 209 | 69.0% | 0.7±0.5 |
| Pelvic edema | 56 | 18.4% | 0.7±0.5 |

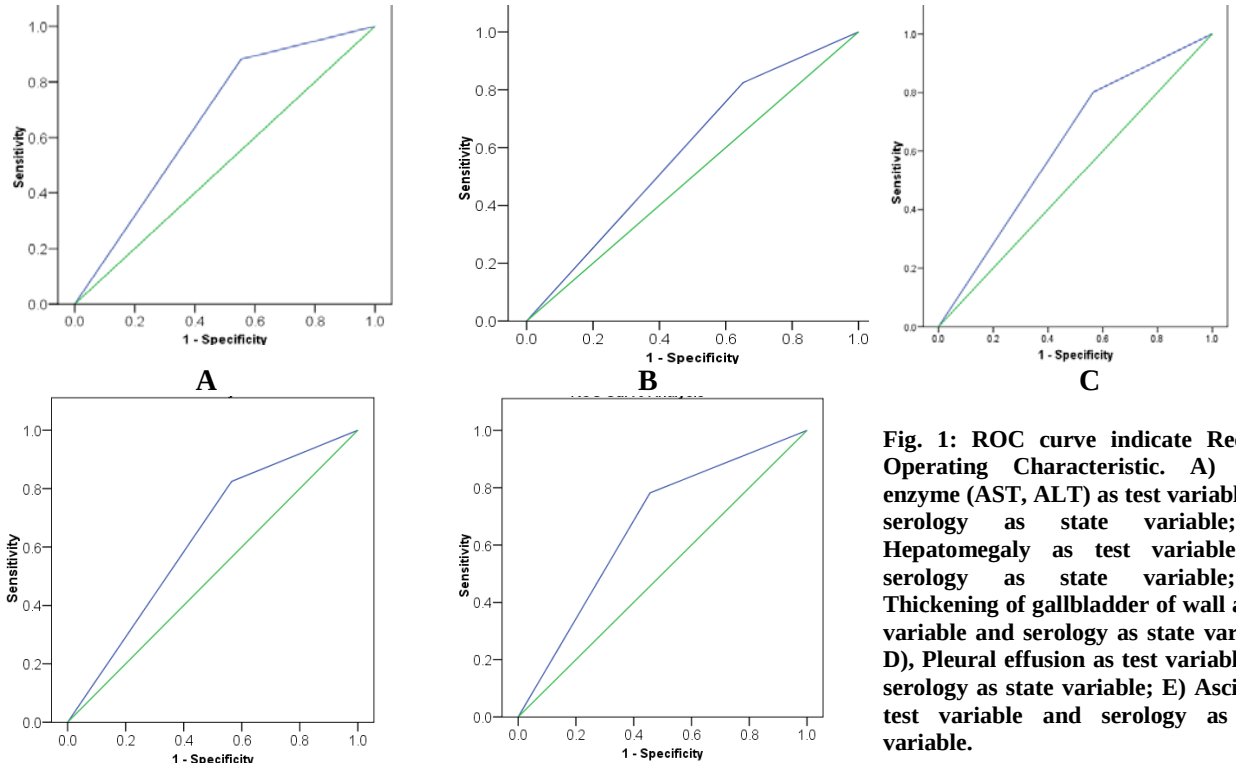


**Fig. 1: ROC curve indicate Receiver Operating Characteristic. A) Liver enzyme (AST, ALT) as test variable and serology as state variable; B) Hepatomegaly as test variable and serology as state variable; C) Thickening of gallbladder of wall as test variable and serology as state variable; D), Pleural effusion as test variable and serology as state variable; E) Ascites as test variable and serology as state variable.**

ROC curve indicate Receiver Operating Characteristic. Its analysis show the area occupied by each variable i.e. hepatomegaly, thickening of gall bladder wall, pleural effusion and ascities. It signify that each variable has P<0.05, so they are significant. Chi square also shows that there is significant association between all variables with serology (Fig. 1).

## DISCUSSION

DHF is an acute febrile illness characterized by headache, retrobulbar and musculoskeletal pain, nausea, vomiting, rash and bleeding diathesis[16,17].

It is diagnosis is suspected on basis of clinical manifestation and laboratory findings, *i.e.* neutropenia (>40,000/L), lymphocytosis, thrombocytopenia (>100,000 /cmm), increase in haemocrit or haemoconcentration above normal i.e. 45% in males and 40% in females and elevation of liver enzymes (AST 36 U/L, ALT 42 U/L), raised APTT (25-39 sec). The presumptive diagnosis can be confirmed by serology in which IgG and IgM are raised. There are several diagnostic techniques to document dengue viral infection, but 2 important are: (a) isolation of virus to determine the serotype of immunoglobulin M-enzyme linked immunoassay or IgM ELISA (b) The later the basic test for serological diagnosis, which detects anti-dengue IgM in patients blood indicating recent Dengue infection. The results obtained are too late to be of any clinical benefit. Therefore additional tools for evaluating patients of DHF are being sought out.

The main pathophysiological change results from variable degree of damage to liver parenchyma provoked by dengue virus leading to increase in liver enzyme. These parameters may be used to access the severity of liver damage[14,16]. Dengue virus also can endothelial damage causing increased capillary permeability and thus plasma leakage into extravascular spaces and serous effusion with high protein content. There is loss of proteins especially albumin leading to hypoproteinemia (total protein 63.80 g/l, albumin 30-50 g/l). Also loss of fluid

from circulation leads to increase in haemocrit. The minimum fluid detected by ultrasonography is 175 ml[17]. Thickness of GB wall above 3 mm on ultrasonography is significant.

The findings of this study has considerable consistency with results of study conducted by Setiawan[18]. He documented that thickening GB wall is one of the pioneer marker in early stages of DHF than other findings on ultrasonography. Alkaline phosphatase increase above normal (30-50 g/L) indicating biliary obstruction. In DHF comparable with his results.

The current study show equivalent and comparable results with those of Keng-Liange WU. He reported acalculous cholecystitis and similar findings of thickened GB wall[19,20]. He documented that the main change is increased vascular permeability, causing plasma leakage and serous effusion with high protein content which than induced thickening of GB wall.

This study supports the association between DHF and alteration of liver biomarkers in which liver enzymes are raised. (AST, ALT) Pancharoen in 2002[21,22] reported similar results. Nguyen et al. in 1977 depicted similar results by documenting that the first target of dengue virus is hepatic parenchymal cells leading to their inflammation[21], hence increasing level of liver biomarkers.

Thulkar documented his sonographic findings in 40 patients which included hepatomegally, thickening of gallbladder wall, pleural effusion[15,23,]. This retrospective study exactly matches the findings of current study.

Luis Angel Villar-Centemo concluded that despite clinical variability, the acute phase of dengue begins with fever i.e. indistinguishable from initial phase of other acute febrile illnesses, but there is early alteration of biochemical markers like AST, ALT, which can predict DHF in patients with acute fever showing equivalent and comparability with his study. In addition to these findings he showed that levels of phospholipase A2 was raised which is correlated with C-reactive protein CRP[12] which are not taken into consideration in this study.

In an area experiencing DF epidemic when sonographic findings show hepatomegaly, thickening of gallbladder wall and pleural effusion in a febrile patient with thrombocytopenia and raised serum transferase level. DHF should always be considered in the differential diagnosis until its disproved.

## CONCLUSIONS

Results of this study depict that early diagnosis of disease can effectively modify the coarse of illness preventing mortality and morbidity in under developed country like ours. We thus aim to propose and validate a simple clinical, biochemical and sonographic prediction in identifying patients at risk.

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