**Clinical, Haematological and Biochemical manifestations among Dengue Patients of Lahore region**

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**Abstract:**

The objective of this study was to assess the clinical manifestations and changes in haematological and biochemical parameters in connection to severity of dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) among dengue patients of Lahore region. Total 345 clinically suspected patients and 20 patients of febrile illness other than dengue (OFI), as control were included in this cross-sectional study, conducted at Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore and Jinnah Hospital Lahore (JHL) from January to December 2013

Total 108 (31.3%) patients were serologically confirmed for dengue infection that were classified as classical DF 81(75%), DHF 22(20.4%) and DSS 5 (4.6%). Mean age was 32.3±12.4 years, which comprises of male 80 (74%) and female 28 (25.9%). Common symptoms for dengue were fever and headache (100%), arthralgia (82%), myalgia (80.5%), retro-orbital pain (68.5%), bleeding tendencies (38%), rash (51%) and vomiting (48%). Thrombocytopenia, (90%), leukopenia (62.5%), elevated transaminases (ALT 56.5%, AST 70.5%), hyponatremia (51.8%) hypokalemia (40.7%) and hypocalcemia (81.4%) were assessed among dengue patient.

It was concluded that bleeding tendencies, retro-orbital pain, rash and vomiting were more frequent in DHF and DSS cases. Thrombocytopenia, leukopenia, deranged haematocrit, raised transaminases, urea, creatinine levels, decreased serum levels of albumin, cholesterol, sodium, potassium and calcium were more commonly associated with severe form of disease i.e., DHF and DSS.

**Keywords:**

Dengue fever (DF), Dengue hemorrhagic fever (DHF), Dengue shock Syndrome (DSS), hematological parameters, biological parameters

**Introduction**

Dengue, a global threat, is expanding in more than 100 countries located in tropical and subtropical regions of the world where climatic conditions favor its expansion (Pai *et al.* 2015). Dengue is caused by the four circulating serotypes of dengue virus namely DEN1, DEN2, DEN3, DEN4, that are transmitted through the bite of female mosquitoes of genus Aedes, mainly *Aedes aegypti* and *Aedes albopictus* species. World health organization (WHO) has classified dengue infection into three classes according to the severity i.e., dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (WHO, 1997).

The first dengue epidemic in Pakistan was reported in 1994 by dengue serotype DENV1 and DENV2 (Chan *et al.*1995). The dengue remerged in Karachi in 2005, and serotype DENV3 was isolated. During 2010-2011, Pakistan faced adverse floods that proved to be the breeding sites for dengue virus. As a result, worst outbreaks with 22,562 dengue cases and 363 deaths have been reported out of which (17493) cases with 290 deaths were reported from Lahore, Punjab alone (Rasheed *et al.* 2013) During year 2013, total 8546 dengue cases with 33 deaths were reported from Pakistan (Fatima *et al*. 2013). Keeping in view the severity of dengue infection in Lahore during the year 2011, and its consistent presence in other parts of the country, comprehensive study was conducted in one of the major tertiary level Hospital to monitor dengue infection severity patterns and its effects on various clinical, hematological and biochemical parameters. Significant alterations in hematological and biochemical parameters can be used as predictor to severity of disease

**Materials and Methods**

This study was conducted at Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore and Jinnah Hospital Lahore. Blood samples from all 345 suspected dengue patients were collected who reported during a period of one year from January to December 2013 to JHL. Blood samples of 20 individuals with febrile illness other than dengue (OFI) were taken as control.

**Inclusion criteria**

According to WHO criteria, suspected dengue patients with more than two days of acute febrile illness with any two or more of the symptoms including headache, retro orbital pain, myalgia, rash, bleeding manifestation, leucopenia, were included in the current study (WHO. 1997).

**Exclusion criteria**

Patients with identified bacterial infection, any other specified chronic infection and only IgG positive were not included in the study (Narayanan *et al*. 2002)

**Data collection technique**

The detailed information including name, age, gender, address, days of fever and previous history of dengue for all 345 suspected patients and 20 control samples was recorded in the study with their consent. Blood sample (10 mL) from each individual was taken and divided into three vacutainers for haematological, biochemical and diagnostic studies. Blood samples for diagnostic and biochemical studies were centrifuged and serum were stored at 4̊C for further analysis within a week. Haematological parameters were performed on the same day.

**Dengue infection Identification**

Patients’ blood were subjected to dengue test as non-structural protein 1 (NS1) antigen and dengue specific IgG/IgM antibodies *in vitro* by standard laboratory procedure as Immunochromatographic test (ICT) using commercially available SD BIOLINE Dengue Duo rapid test dengue diagnostic kit (Standard Diagnostic Inc; Seoul, Korea) and by Enzyme linked immunosorbent assay (ELISA) using commercially available kits for NS1 antigen, IgM and IgG antibodies (Diagnostic Automation/Cortez Diagnostics Inc, California, USA). All samples were analyzed according to manufacturer protocol.

Blood samples confirmed serologically positive by both ICT and ELISA method were divided into three groups DF, DHF and DSS revealing severity of disease as per WHO criteria and were included for further evaluation(WHO. 1997).

**Haematological parameters**: Complete blood count including hemoglobin (Hb), hematocrit (HCT), total leucocyte count (TLC) and platelet count in blood samples of dengue patients were performed on Hematology analyzer “Sysmex KX-21” (Sysmex America Inc, Lincolnshire, Illinois, USA).

**Biochemical parameters:**  Biochemical markers determined for liver function test (LFT) were, total protein (TP), albumin, bilirubin (Bili), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP). Parameters included in renal function tests (RFTs) were urea, creatinine (Cr) and uric acid (UA) while parameters estimated for lipid profile were cholesterol, triglyceride (TG), high density lipoprotein- cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c). To determine heart involvement, cardiac enzymes including creatine kinase (CK), lactate dehydrogenase (LDH), and creatine kinase-MB (CK-MB) were estimated. Serum electrolytes including calcium (Ca), phosphorus (P), sodium (Na) and potassium (K) were also assessed. Serum Ca and P were estimated by using AU kits (Beckman Coulter Inc. Brea California, USA) while serum Na and K electrolyte were analyzed by ion selective electrode method using the “EasyLyte analyzer” (Medica Corporation, Bedford, USA). Biochemical parameters were estimated on automatic chemistry analyzer “Beckman Coulter AU 480” by standard AU reagent (Beckman Coulter Inc. Brea California, USA) according to the manufacturer instructions.

Calibration of each chemistry parameter was performed according to the instructions of instrument. To check the accuracy of analysis, quality control sera analyzed for all chemistry parameters and results were within range.

**Statistical Analysis**

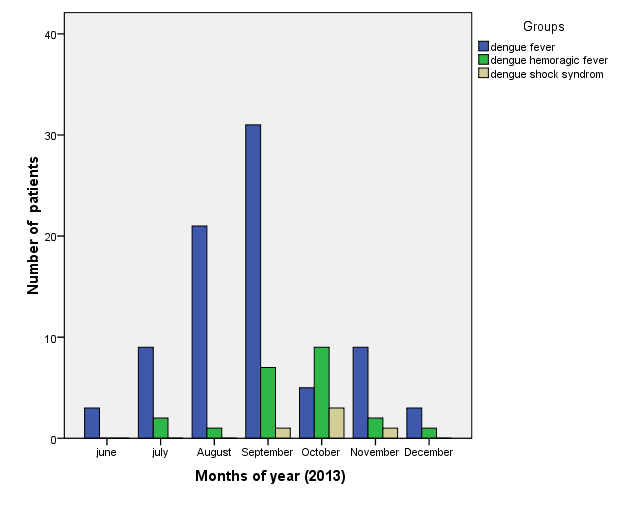
The SPSS software version 21 was used for statistical analysis. Continuous variables were expressed as mean ± SD and categorical variables as frequency and percentages. Association between clinical features and severity was analyzed by Chi-square and Fisher’s Test. ANOVA was used to determine the relationship of haematological and biochemical parameters with severity of dengue fever. P-Value *P*<0.05 was considered to be statistically significant.

**Results:**

Blood samples of 345 suspected dengue patients and 20 control were subjected to serological testing. 114 (33%) samples were confirmed positive by ICT method while 110 (31.8%) were found to be positive by ELISA. Total 108 (31.3%) samples confirmed positive by both the ICT and ELISA techniques were utilized for further studies.

The mean age of the patients in this study was 32 years with a range of 11-70 years. The maximum number of patients 55 (50.9%) belonged to age group 11-30 years. Secondly most frequently exposed were 44(40.7%) patients of age group 31-50 years. Least exposed were 9(8.3%) patients of age group 51-70 years. Out of 108 serologically positive patient 28 (25.9%) were females and 80 (74%) were male. Among the positive cases 81 (75%) patients were classified as DF, 22 (20.4%) patients as DHF and 5 (4.6%) as DSS.

Dengue cases were not reported through January to June, then increased through the months of July to September (cases 74- 68.5%) and then declined through months of October to December. Fig-1 shows the prevalence of dengue from June to December 2013.



**Figure 1:** Prevalence of dengue during the months of June, to December, 2013

Patients were presented with different clinical features such as fever, headache, chills, arthralgia, myalgia, vomiting, diarrhea, and maculopapular rash. Analysis of symptoms demonstrated that all patients presented with fever and headache during their first hospital visit. Myalgia, arthralgia, retro-orbital pain, chills, abdominal pain, rash, vomiting and bleeding manifestation were significantly more frequent in patients of DHF and DSS (Table 1).

**Table:**1 Comparison of clinical parameters between dengue fever, dengue hemorrhagic fever and dengue shock syndrome

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Symptoms | Total dengue positive patients  number(%age) | DF number (%age) | DHF number (%age) | DSS number (%age) | Control  number (%age) | P value |
| Fever | 108 (100) | 81 (100) | 22 (100) | 5 (100) | 20 (100) | - |
| Myalgia | 87 (80.5) | 64(79.0) | 19 (86.4) | 4 (80) | 9 (45) | .008 |
| Headache | 108 (100) | 81 (100) | 22 (100) | 5 (100) | 20(100) | - |
| Chills | 84 (77.7) | 63 (77.7) | 16 (72.7) | 5 (100) | 11 (55) | .104 |
| Retro orbital pain | 74 (68.5) | 51 (63) | 18 (81.8) | 5 (100) | 4 (21.1) | .000 |
| Abdominal Pain | 44(40.7) | 21 (25.9) | 19 (86.3) | 4 (80) | 4 (20) | .000 |
| Arthralgia | 89 (82) | 65 (80.2) | 20 (90.9) | 4 (80) | 6 (30) | .000 |
| Hemorrhagic manifestations | 42 (38) | 16 (19.8) | 21 (95.5) | 5(100) | 0 (0) | .000 |
| Rash | 56 (51) | 35 (43.2) | 17 (77.3) | 5 (100) | 0 (0) | .000 |
| Vomiting | 52 (48) | 31 (38.3) | 17 (77.3) | 4 (80) | 6 (30) | .000 |

**Haematological parameters**

Hematocrit was raised in dengue patients as compared to controls. Highest mean HCT level was reported in DSS patients. In this study, raised HCT level >45% was observed in 34 (42.5%) male and >40% was seen in 9 (32.I%) female patients. Low levels (<40%) of HCT was observed in 24 males and level (<35%) of HCT was seen in 15 (53.5%) females. Leukopenia (leucocyte count< 4000 cells/µL) and thrombocytopenia (Platelet count<100000 cells/µL) were the most commonly observed hematological features in dengue patients. Severe thrombocytopenia (Platelet count <25000 cells/µL) was reported in 4 (18.1%) patients of DHF and 4 (80 %) DSS group, while mild thrombocytopenia (platelet count more than 50000 cells/µL) was present in 70(86.4%) of the DF patients. Table 2 clearly demonstrate the variation in haematological parameters due to DF, DHF and DSS.

**Table 2:** Variation in Haematological parameters due to DF, DHF and DSS

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **DF (n=81)** | **DHF (n-22)** | **DSS (=5)** | **Control (n-20)** | **p- value** |
| Hemoglobin (g/dL) | 12.7± 1.9 | 13.03± 2.1 | 14.3±0.54 | 13.4± 1.55 | 0.119 |
| Hematocrit (%) | 42.2±5.77 | 43.9±6.53 | 46.4±5.62 | 35.29±5.88 | 0.000 |
| Total leucocyte (cells/µL) | 3248±762 | 3097±895 | 2660±594 | 7024±1409 | 0.000 |
| Platelet Count(cells/µL) | 73741±19203 | 33227±11803 | 19560±3642 | 327544±67556 | 0.000 |

**Biochemical parameters.**

The levels of transaminases were significantly high, while serum levels of total protein and albumin were significantly low among dengue groups. The increased AST/ALT ratio in these studies differentiated dengue infection from other acute hepatitis caused by hepatitis A, B and C viruses. Increased urea and creatinine levels were estimated in DSS patients (Table 3). Patients with other febrile illness had low levels of transaminases than DF, DHF and DSS patients. The estimated levels of total cholesterol, HDL-c and LDL-c in dengue groups (DF, DHF and DSS) were significantly low, while in patients with OFI the serum levels of total cholesterol, HDL-c and LDL-c were high. Conversely, TG levels were elevated in dengue patients as compared to Control group. The patients of DSS group had lowest HDL-c level that indicated severity of disease. Cardiac enzymes (CK, CK-MB and LDH) levels were raised in dengue groups as compared to control due to myocardial cell injury caused by dengue infection. The estimated levels of Na+, K+, and Ca++ were significantly decreased in DHF and DSS patients as compared to control patients (Table 3).

Table 3**:** Variations in Biochemical tests levels due to DF, DHF and DSS

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Biomarkers** | **Reference range** | **DF (n=81)** | **DHF (n=22)** | **DSS (n=5)** | **Control (n=20)** | **P** |
| **Liver profile** | | | | | | |
| AST(U/L) | 11-50 | 94.3±95.0 | 145±128 | 301±109 | 31.7±10.2 | 0.00 |
| ALT(U/L) | 11-50 | 73.6 ±78.8 | 104 ±78.3 | 155.4±94.8 | 26.5±11.6 | 0.00 |
| ALP(U/L) | Up-to 300 | 141±65.8 | 181.4±75.3 | 192.2±61.8 | 159.6±70.2 | 0.72 |
| Bilirubin(mg/dL) | 0.4-1.2 | 0.96±0.82 | 1.37±.83 | 1.80±0.1.3 | 0.64±0.20 | 0.00 |
| Albumin (g/dL) | 4.5-5.2 | 3.6±0.59 | 3.1±0.49 | 2.6±0.5 | 4.4±0.79 | 0.00 |
| Protein (g/dL) | 6.6-8.5 | 6.8±0.96 | 6.1±0.74 | 5.7±0.63 | 7.7±0.64 | 0.00 |
| **Renal profile** | | | | | | |
| Urea (mg/dL) | 11-43 | 24.4±6.4 | 47±41.7 | 71.0±45 | 23.2±5.9 | 0.00 |
| Creatinine (mg/dL) | .0.7-1.1 | 0.64±0.22 | 1.03±1.18 | 1.9±1.21 | 0.68±0.15 | 0.00 |
| Uric Acid (mg/dL) | 3.5 -7.2 | 4.5±0.92 | 4.1±1.4 | 4.0±0.66 | 4.1±0.99 | 2.08 |
| **Lipid profile** | | | | | | |
| Cholesterol (mg/dL) | 180-200 | 140±34.7 | 121.9± 28 | 112± 5.7 | 199 ±31.5 | 0.00 |
| Triglycerides (mg/dL) | < 150 | 213±54.8 | 235±75.2 | 259± 59.3 | 193 ±60.5 | 0.03 |
| HDL-c (mg/dL) | 40-60 | 25±6.5 | 23.9±4.1 | 19.8± 0.83 | 36 ±6.4 | 0.00 |
| LDL-c (mg/dL) | 100-129 | 92.8±27 | 84.3± 22.6 | 80.6± 2.87 | 12 9±28.6 | 0.00 |
| VLDL-c (mg/dL) | <35 | 41.6±12.2 | 46.6±15.1 | 51.6±11.8 | 38.6±12.0 | 0.25 |
| **Cardiac profile** | | | | | | |
| CPK IU/L | 24-171 | 225±199 | 274±89.5 | 378.6±200 | 137±38.4 | .148 |
| CK-MB IU/L | Up-to 25 | 36±4.7 | 46±26.8 | 78±45.1 | 14±5.5 | .029 |
| LDH IU/L | 208-378 | 341±134 | 467±149 | 504±83.1 | 265±106.7 | .007 |
| **Electrolyte** | | | | | | |
| Sodium (mmol/L) | 135-147 | 132±6.0 | 128.1±3.8 | 124.8±4.0 | 135.1±6.1 | .00 |
| Potassium (mmol/L) | 3.5-5.2 | 4.6±1.28 | 3.0±0.35 | 2.66±0.26 | 4.66±0.79 | .00 |
| Calcium (mg/dL) | 8.8-10.5 | 8.5±0.74 | 7.38±.70 | 6.8±0.39 | 9.0±0.92 | .00 |
| Phosphorus (mg/dL) | 2.4 4.5 | 3.81±2.3 | 3.34±1.2 | 2.8±0.60 | 3.84±0.53 | 0.57 |

**DISCUSSION**

This study was designed to develop a comprehensive picture showing the impact of dengue infection on various clinical, haematological and biochemical parameters in blood samples of patients. Prevalence of dengue infection in male population was more as compared to female population. The male population in Pakistan, being expected to work outside are more exposed to mosquitoes than female. Moreover, females in Pakistan cover themselves with clothes properly as compared to North America and Vietnam where equal populations of males and females were reported to be affected by dengue infection(Gunther *et al*. 2009) Findings of this study was supported by (Gandhi and Shetty 2013).

Prevalence of dengue infection was higher in young (11-50 years) as compared to elderly people (50-70 years). High frequency of disease in young group was due to large population size in this age category and more exposure due to their outdoor activities. Other studies also supported that the intensity of dengue was higher in young group as compared to the old age group(Fatima *et al*. 2016; Gunther *et al.*2009) Higher number of patients were observed in July to October period. Prevalence of dengue was associated with the monsoon and post monsoon season due to increase in breeding sites of mosquitoes, in accordance to other studies (Fatima *et al*. 2016)

The most common clinical features observed in this study were fever, headache, myalgia, and arthralgia, in consistence with other studies(Namvongsa *et al*. 2013) Clinical feature such as persistent vomiting, bleeding, severe abdominal pain and petechiae were associated with severe forms of dengue as DHF and DSS and similar findings were reported in another study conducted in India(Bhaskar *et al*. 2010)

Haematocrit (HCT) levels in non-dengue patients were significantly low when compared to dengue patients. Highest mean HCT levels were estimated in DSS. The rise in HCT level was associated with plasma leakage due to increase in vascular permeability. A 20% rise in HCT was documented previously as cut off for diagnosis of DHF but in this study, elevation in HCT levels were less than expected and similar findings of less rise in HCT were reported previously (Bhaskar *et al*. 2010; Jain *et al*. 2013). This shows there is a need to develop new recommendations for HCT levels. Most frequently observed leukopenia and thrombocytopenia in dengue patients was due to bone marrow suppression and binding of dengue antigens to platelets (Jameel *et al*. 2012).

Hematological abnormalities (thrombocytopenia) were found in 90% of the patients. This is in agreement with the previous studies (Gunther *et al*. 2009).Karoli diagnosed thrombocytopenia in 89% patients and Yadav reported in 92% patients (Karoli *et al*.2012; Yadav 2018).

This study demonstrated the elevated transaminases in 94% of the dengue patients. These results are in agreement with the previous reports from Singapore, Vietnam and India showing 86, 97 and 97.5% patients with elevated transaminases levels (Lee *et al*. 2012; Hien *et al*. 2010; Gupta *et al.* 2014). Brazil had reported elevated transaminases level in relatively low (64%) dengue cases (Souza *et al*. 2004). AST/ ALT levels tend to be greater and this differ from the pattern in viral hepatitis. Hyperbilirubinemia was significantly common in DSS and these results are consistent with the reports from India 9Chhina *et al*. 2008). Serum albumin levels were recorded significantly low in dengue patients, consistent with increase in severity of disease with least levels recorded in DSS. This decrease can be correlated with the increasedplasma leakage and increased vascular permeability in DSS. Decreased albumin levels were also reported by Villar-Centeno and his college’s (Villar *et al*. 2008).

Renal impairment was associated with the severity of disease as raised urea and creatinine levels were recorded in 18% DHF and 60% DSS. These results are in accordance to previous reports (Lee *et al*. 2006; Abboud 2015).

Lipid profile examination elaborated the decrease in total cholesterol, HDL-c and LDL-c and increase in triglyceride and VLDL-c in DF, DHF and DSS as compared to control group. Mechanism behind this lipoprotein changes is that lipid metabolism and dengue induced cytokine production are interlinked. Increased levels of triglycerides are the result of increased lipolysis and de novo fatty acid synthesis in liver due to enhanced activity of specific enzyme. Finding of this study were in line with the result of previous studies (Van Gorp *et al*. 2002). Alterations in lipid profile levels could be used as prognostic markers to predict clinical outcome.

Cardiac profile described the increase in levels of CPK generally whereas elevated CK-MB and LDH levels are correlated with the severity of disease. These results are supported by the findings of Villar and his colleagues (Villar *et al*. 2008)

Current study demonstrated low levels of sodium, potassium and calcium in DHF and DSS as compared to DF and control. This situation might be due to plasma leakage in severe dengue condition, with results supported by previous reports (Mekmullica *et al*. 2005; Lumpaopong *et al*. 2010)

**Conclusion**

On the basis of above findings, it can be concluded that the haematological parameters, thrombocytopenia, leukopenia and raised HCT were relevant to severity of disease. Clinical recovery monitoring is dependent on hematological parameters. The hematological profile can be used as screening tool to assess early therapeutic response. This study suggested that some biochemical alterations as raised transaminases, urea, creatinine and triglyceride levels and decreased serum levels of albumin, sodium, potassium, calcium, cholesterol, HDL and LDL can be used as predictor of dengue complication. Patients with deranged parameters should be treated with extra care to avoid complication.

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