Malaysian J Pathol 2015; 37(3) : 247 – 251

ORIGINAL ARTICLE

Haematological and biochemical markers as predictors of dengue infection

RASHMI MV *MD* and HAMSAVEENA* *MD*

*Departments of Pathology and *Biochemistry, Sri Siddhartha Medical College, Tumkur, India*

Abstract

Dengue is a viral infection which has become a serious problem in recent years. It is a major cause of mortality and morbidity. The present study is a prospective, hospital-based, observational study done from August 2014 to October 2014. The objective of our study was to consider whether dengue infection can be suspected based on haematological and biochemical findings. The study included 100 patients positive for dengue infection. Complete hemogram, transaminases for liver injury, blood urea and serum creatinine levels for renal assessment were performed for these patients. The most common haematological findings were thrombocytopenia, leucopenia, an increase in the mixed cell fraction of the leucocytes and the presence of reactive lymphocytes. Biochemical parameters like the aminotranferases, blood urea and serum creatinine levels were significantly raised. Hence, a platelet count of <100,000 cells/µl, leucopenia of <4000cells/µl and aspartate aminotransferase levels of >82.2 U/L can be considered as predictors of dengue infection.

Keywords: dengue fever, hematological parameters, biochemical parameters

INTRODUCTION

Dengue is an emerging tropical viral infection caused by an ssRNA virus of the flaviviridae family and transmitted by the bite of *Aedes aegypti* and *Aedes albopticus* mosquitoes. WHO estimates 50-100million cases of dengue each year. More than three hundred thousand cases of dengue hemorrhagic fever (DHF) are diagnosed each year resulting in 24,000 deaths per year. Dengue fever has been reported from India over a long time but DHF was first reported in 1963 from Calcutta.[1,2]

Dengue is a self-limiting infection which causes a spectrum of illness ranging from no symptoms to life threatening DHF/dengue shock syndrome. It shares some symptoms with other infectious diseases, most of which go undiagnosed and under reported.[3]

It is a major cause of morbidity and mortality in tropical and subtropical regions comprising more than 100 countries. Two-fifths of the world's population or 2500 million people are now at risk for dengue. The global prevalence of dengue infection has increased dramatically in the recent decades, particularly in the Americans, Western Pacific and South-East Asian regions.[4]

Early diagnosis and aggressive fluid replacement therapy with good nursing care can reduce the fatality rates to 1% or less. Serological testing is currently the standard diagnostic practice for confirmation of dengue infection.[5,6]

The present study was undertaken to consider whether dengue viral infection can be predicted based on haematological parameters and biochemical tests for the liver and renal functions.

MATERIAL AND METHODS

One hundred (100) of proven dengue cases were included in the present study. Ethical clearance for the study was obtained from the institutional ethical committee. The subjects of this study were patients who attended the out-patient department or were admitted as in-patients in our institution. All patients had fever of at least 3 days duration and the blood investigations were performed on the 3rd to the 5th day of illness. All patients who tested positive for dengue infection were included in the study. Patients with fever but were negative for dengue testing were excluded from the study.

Address for correspondence and reprint requests: Dr. Rashmi MV, Professor, Department of Pathology, Sri Siddhartha Medical College, Tumkur-572104, India. Phone no.: 09449666400. E-mail: rashmiarava@yahoo.com

Test parameters
Haematological and serological investigations were studied in such patients. 5ml of blood was drawn, 2 ml of blood in EDTA vacutainers for complete hemogram and 3ml of blood in plain vacutainers for serological investigations.

The test for dengue was done using a rapid solid phase immune-chromatographic test for the qualitative detection of NS1 antigen and differential detection of IgM and IgG antibodies to dengue virus in human serum.

The hematological investigations included a complete hemogram which included complete blood counts and peripheral smear. The blood counts were performed on the fully automated hematology analyzer with a three part differential. The peripheral smears were stained with Leishman stain and studied by a pathologist. The biochemical investigations included aminotransferases estimation for liver involvement and blood urea and serum creatinine for the renal function. The tests were performed on automated biochemistry analyzer.

RESULTS

The majority of our patients were diagnosed with dengue in the post monsoon months of September and October. Most of the patients presented with clinical symptoms of fever, myalgia and headache for 3 to 5 days. On examination, a rash was observed in very few cases especially the fair-skinned individuals. Occasional patients even complained of running nose and petechiae. These patients were initially subjected to investigations including complete hemogram, dengue, malaria and typhoid. Only those patients tested positive for dengue were considered for the present study. Such patients were further tested for liver and renal involvement.

Demographic profile
In the present study, there were 62 males and 38 females. The ages ranged from an infant of 2 months to an old man of 80 yrs. However, the largest proportion (26%) of patients was in the age group of 20-29 yrs. 21% and 18% of patients were in the 30-39 yr and 10-19yr category respectively. The average age at presentation was 28.6 yrs.

Laboratory findings
Of the 100 cases included in this study, the majority showed only antigen NS1 positivity (Table 1). Very few patients were positive for only antibodies or a combination of both antigen and antibodies.

The most common haematological changes were thrombocytopenia, leucopenia and an increase in mixed cell fraction of the WBC cell distribution. The haemoglobin levels ranged between 7.9-19.2g/dl with an average of 10.8g/dl. The total WBC count was less than 4000/µl in 60% of cases. The mixed cell population which includes monocytes, eosinophils and basophils in the three part differential by the automated cell counter was found to be raised in 51% of the cases. 80% of the cases had platelet count of <150,000/µl of which (29%) had platelet counts of 40,000-80,000/µl (Table 2).

Haematocrit levels were raised in 23% cases. The RBC count on average was 4.54 million/µl. The peripheral smears in all these cases were studied by a pathologist. 80% of the smears showed reactive lymphocytes which appeared as large monocytoid cells which caused the increase in mixed cell fraction of the differential count. However, we noticed that these cells were very pale staining even when the rest of the smear was well stained and leucopenia was also appreciated on the smear.

Liver function tests were performed and all parameters were normal except for aminotranferases which were raised in 58% of cases (Table 3).[7] The average aspartate aminotranferase (AST) level was 82.2U/L and alanine aminotransferase (ALT) was 57.6U/L. The levels of aminotranferases were much higher than normal values. The blood urea and serum creatinine levels were raised in 23% of the patients and were normal in the rest of the patients. The average blood urea level was 40.2 mg/dl and that of serum creatinine was 1.5 mg/dl.

TABLE 1: Pattern of dengue antigen and antibody positivity

Antigen/antibody	No. of cases
NS1+	87
NS1+, IgM+	04
NS1+. IgG+	02
IgM+, IgG+	02
IgM+	02
IgG+	03
Total	100

TABLE 2: Distribution of platelet counts in the 100 study subjects

Platelet count/µl	No. of cases
0-19,000	04
20,000-39,000	24
40,000-79,000	29
80,000-99,000	11
100,000-150,000	12
>150,000	20

DISCUSSION

In our study the majority of cases occurred in the post monsoon season. Hakim *et al* also have reported the maximum number of cases in the month of October. This could be due to the ambient temperature and humidity in the environment during this period, which is supposedly ideal for mosquito breeding.[8]

The ages of the patients ranged from 2 months to 80 yrs. The average age at presentation was 28.6 yrs, and that largest proportion of patients was in age group of 20 – 29 yrs. Arshad *et al* also reported an increased incidence of dengue in the age group of 21–30yrs. Rusmavathi *et al* found the maximum number of cases in the 13 – 39 yrs group and in the study by Khan *et al*, the average age of presentation was 35 yrs.[4-6]

Males constituted 68% of the study cases with a male to female ratio of 1.6: 1. In other studies also by Arshad *et al*, Gireesh *et al* and Dutta *et al*, males outnumbered females. In the study by Hakim *et al*, maximum suspected cases were males. This has been attributed to the Asian culture whereby males spend more time outside their houses and are more likely to be exposed to mosquitoes compared to females.[3,5,8-10]

In the present study, the common haematological findings were thrombocytopenia, leucopenia and an increase in the mixed cell fraction. The peripheral smears showed reactive lymphocytes. The serum levels of AST and ALT were also raised in a substantial proportion of cases. Wilder Smith *et al* concluded, after multivariate analysis, that three laboratory features, when present, are highly predictive of dengue fever. These include platelet count of $<140 \times 10^9$/L, white blood cell count of $<5 \times 10^9$/L and aspartate aminotranferases level of >34IU/L. A combination of these 3 laboratory tests has a sensitivity of 75% and specificity of 100%.[11]

The incidence of thrombocytopenia in our study, where the platelet count was less than 150,000/µl was 80%. The majority of our patients had counts between 40,000 – 80,000/ µl. In the study by Banerjee *et al*, the platelet count ranged between 40,000 – 100,000 cells/c.mm in 19% of cases. Arshad *et al*, found a platelet count of less than 50000/c.mm was in 78% of patients. Similarly, Rusmavathi *et al* and Dutta *et al,* reported thrombocytopenia in 77.8% and 71.3% of their patients respectively. The reduction

TABLE 3: Mean values and significance of demographic and laboratory findings[7]

Parameter	Mean value	Normal values[7]
Age	28.6 yrs	
Sex	M:F:: 1.6:1	
Hb (g/dl)	10.8	M: 14 – 17.5 F: 12.3 – 15.3
RBC count ($x10^6$/µl)	4.54	M: 4.5 - 5.9 F: 4.5 - 5.1
WBC count ($x10^3$/µl)	3.73	4.4 -11
Platelets (µl)	97000	150000 – 450000
Hematocrit (%)	38.9%	M: 39 - 49 F: 33 - 43
MCV(fL)	73.2	80 - 96
MCH(pg)	25.06	27.5– 33.2
MCHC(g/dl)	30.48	33.4 – 35.5
AST (U/L)	82.2	8 – 33
ALT (U/L)	57.66	4 – 36
BUN (mg/dl)	40.2	8 – 23
Creatinine (mg/dl)	1.5	0.6 – 1.2

in platelet count was attributed to depression of the bone marrow, direct infection of the megakaryocytes by the virus and to the presence of antibodies to platelets. In the review article by Lei *et al*, thrombocytopenia was attributed to autoantibodies which induce platelet lysis via complement activation. The study also reported the cross-reactivity of antibodies directed towards dengue virus proteins, especially NS1 and platelets suggesting the pathogenic role of anti-platelet autoantibodies during dengue virus infection. Khan *et al* concluded that thrombocytopenia was a persistent finding in dengue, however, an absence of thrombocytopenia should not rule out the possibility of dengue infection.[1,3,5,6,11,12]

In our study, 60% of patients had a total leucocyte count of less than 4000/ µl. Arshad *et al*, reported that 49% of patients had leucopenia. Jameel *et al* found WBC count of less than 3000 cells/c.mm in 56.6% of cases. However, in the studies by Rusmavathi *et al* and Dutta *et al*, the incidence of leucopenia was less and formed 33.3% and 30% of the cases respectively. Lei *et al* suggested that dengue infected patients were usually leukopenic and characterized by a reduction in the absolute number of neutrophils and monocytes. This was attributed to a decrease in the proliferative responses of peripheral blood mononuclear cell (PMBC) to mitogen and recall antigen and to phytohaemagglutinin (PHA) stimulated T-cell responses of dengue patients being impaired.[3,5,6,12,13]

The mixed cell fraction of the differential count which includes the monocytes, eosinophils and basophils was increased in 51% of our cases. In none of the articles referred by us, do any of the authors mention regarding the increase in the mixed cell fraction.

The hematocrit levels were raised in only 23% of cases in our study. Jameel *et al* also reported a comparable incidence of 26% in their study. However, Rusmavathi *et al* did not find any hemoconcentration in any of their patients. Furthermore, in our study as well as in the study by Rusmavathi *et al*, the time of sampling in relation to the course of illness was not documented. This might have contributed to the discrepancy.[6,13]

The peripheral smears were studied in all our cases. Atypical lymphocytes were seen in a large proportion. These cells were large and monocytoid. Rusmavathi *et al* reported atypical/ activated lymphocytes in 85.1% of cases and an even higher percentage of 93% was reported by Jameel *et al*. In the review article by Lei *et al*, it was found that in dengue viral infection, an early monocytosis, transient inversion of CD4/ CD8 ratio, increased atypical lymphocytes and depressed T- cell proliferation were observed. These were proposed to contribute to delay in viral clearance and trigger overproduction of cytokines and auto-antiplatelet antibodies.[6,12,13]

The aminotranferase levels were raised in 58% of our cases. The levels of AST were higher than ALT levels. Banerjee *et al* reported an increase in AST and ALT levels in 60% of cases and Dutta *et al* have reported AST was raised in 68.5% and ALT in 39.2% of cases. Dengue virus is hepatotrophic and its antigen has been detected in hepatocytes. Elevated serum transaminases levels have been found in dengue patients and the degree of AST levels elevation correlates with that of hemorrhage. In dengue viral hepatitis, the level of AST is higher than ALT with a ratio of around 1 – 1.5, while other types of virally induced hepatitis have higher ALT levels than AST. Dutta *et al*, have suggested liver injury due to direct infection of hepatocytes and Kuffer cells.[3] Lei *et al* found that patients with dengue virus infection have increased RANTES serum levels compared to those with other viral infections.[12] As RANTES is a chemokine capable of recruiting lymphocytes and NK cells to inflammatory sites, whether liver damage in dengue virus infection is an indirect effect of RANTES or a direct effect of virus replication needs further evaluation.

Conclusion

We conclude that an early diagnosis of dengue viral infection can be predicted based on thrombocytopenia of <100,000/µl, leucopenia of < 4000/µl and an increase in AST levels of > 82.2 U/L. The other supportive evidences are an increase in the mixed cell fraction of the differential counts and reactive lymphocytes on peripheral smears. These findings are noteworthy as an early diagnosis and aggressive fluid replacement therapy with good nursing care can reduce fatality rates to 1% or less.

REFERENCES

1. Banerjee M, Chatterjee T, Choudhary GS, Srinivas V, Kataria VK. Dengue: A clinicohematological profile. MJAFI. 2008; 64(4): 333-6.
2. Prathyusha CV, Srinivas Rao M, Sudarsini P, Uma Maheswar Rao K. Clinico-hematological profile and outcome of dengue fever in children. Int J Curr Microbiol App Sci. 2013; 2(10): 338-46.
3. Dutta P, Khan SA, Borah J, Mahanta J. Demographic and clinical features of patients with dengue in

Northeastern region of India: A retrospective cross-sectional study during 2009-2011. J Virol Microbiol. 2012. DOI: 10.5171/2012. 786298.

4. Khan AH, Hayat AS, Masood N, Solangi NM, Shaikh TZ. Frequency and clinical presentation of dengue fever at tertiary care hospital of Hyderabad/ Jamshoro. JLUMHS. 2010; 9(2): 88-94.
5. Arshad I, Malik FA, Hussain A, Shahida A. Dengue fever clinico-pathologic correlations and their association with poor outcome. Professional Med J. 2011; 18(1): 57-63.
6. Rusmawati I, Asma Hanim H, Naznin M, Salman MS, Norlelawati AT. A descriptive study of blood films of patients serologically positive for dengue in Hospital Tengku Ampuan Afzan, Kuantan. Int Med J Malaysia. 2010; 9(2): 35-7.
7. Lehmann HP, Henry JB. SI units. In: McPherson RA, Pincus MR, editors. Henry's clinical diagnosis and management by laboratory methods. 21st ed. Philadelphia: Saunders Elsevier; 2009. p. 1404-18.
8. Hakim ST, Tayyab SM, Qasmi SU, Nadeem SG. An experience with dengue in Pakistan: An expanding problem. Ibosina J Med BS. 2011; 3(1): 3-8.
9. Achalkar GV. Dengue: A clinic-pathological study of 50 cases. J Evolution Med Sci Dental Sci. 2013; 2(48): 9380-5.
10. Ahmed S, Arif F, Yahya Y, *et al*. Dengue fever outbreak in Karachi 2006 - A study of profile and outcome of children under 15 years of age. J Pak Med Assoc. 2008; 58(1): 4-8.
11. Wilder-Smith A, Earnest A, Paton NI. Use of simple laboratory features to distinguish the early stage of severe acute respiratory syndrome from dengue fever. Clin Infect Dis. 2004; 39(12): 1818-23.
12. Lei HY, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC. Immunopathogenesis of dengue virus infection. J Biomed Sci. 2001; 8(5): 377-88.
13. Jameel T, Mehmood K, Mujtaba G, Choudhry N, Afzal N, Paul RF. Changing haematological parameters in dengue viral infections. J Ayub Med Coll Abbottabad. 2012; 24(1): 3-6.