

Research Article**Hematological and Serological Test Profile in Dengue, Dengue Hemorrhagic Fever and Dengue Shock Syndrome in Bathinda Region of Punjab**Geetika Dhir¹, Tanish Dhir², Vijay Suri³, Dinesh Dhir⁴, Kavin Khatri⁵¹Consultant pathologist, civil hospital, Ferozepur, India. Pin code- 152002.²Intern, Adesh institute of medical Sciences and medical research, Bathinda, India. Pin code- 151001.³Professor, department of pathology, Adesh institute of medical Sciences and medical research, Bathinda, India. Pin code- 151001.⁴Director, Dhir hospital, outside Zira gate, Ferozepur. Pin code- 152002.⁵Assistant professor, department of orthopaedics, GGS Medical College and hospital, BFUHS, Faridkot, Punjab, India. Pin code-151203.***Corresponding author**

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Abstract: Dengue virus is the most common cause of Arbo virus disease in the world causing an estimated 100 million cases of dengue fever, 250,000 cases of dengue hemorrhagic fever and 25,000 deaths per year. The disease warrants early diagnosis for effective treatment to prevent mortality. The objective was to analyze the hematological parameters for early diagnosis of dengue. The parameters studied included hemoglobin, total leucocyte count, differential leucocyte count, platelet count and leishman stained peripheral smear examination after confirming it by antigen and serological tests. Thrombocytopenia was further correlated in association with NS1 Antigen (Ag) & IgM/IgG antibody positivity. Seasonal variations in Dengue positive cases were also observed. Out of 425 dengue positive patients proven by immuno chromatographic method; the common laboratory findings was thrombocytopenia, of which 101 (31.96 %) patients had a mild thrombocytopenia; 143 (45.25%) had moderate thrombocytopenia, and 72 (22.78%) had severe thrombocytopenia. Leucopenia was found in 37.8% of patients and 33.95% of patients had relative lymphocytosis. Reactive/atypical lymphocytes were seen in 11.05% of patients. Also noted was a raised hematocrit, seen in 15% of the patients. Inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly. Thrombocytopenia correlates well when NS1 and IgM are detected simultaneously. In the present study, thrombocytopenia was the commonest associated findings. The other laboratory parameters like hemo concentration, leucopenia and atypical lymphocytes were also noted. They can be used as indicators for early diagnosis of dengue fever.

Keywords: Dengue, Thrombocytopenia, Atypical lymphocytes, Dengue diagnosis, Dengue epidemiology, Dengue immunology, Severe Dengue diagnosis, Severe Dengue epidemiology

INTRODUCTION

The name dengue is derived from the Swahili phrase “Ki Denga Pepo”, meaning a seizure by a demon [1]. Dengue fever is one of the most common arthropod borne viral disease and is prevalent in the tropical and subtropical regions. It is also known as break bone fever and is characterized by biphasic fever, headache, myalgia, rash, arthralgia, leucopenia and varying degrees of thrombocytopenia [2]. It is being recognized as an important emerging infectious disease. Its incidence has been estimated to have increased by 30 folds in the last 50 years. Each year an estimated 100 million cases of dengue fever and about 2.5- 5 lakh cases of dengue hemorrhagic fever are reported to the World Health Organization (WHO) [3]. Dengue is now endemic in over 100 countries with dramatic increase in

geographical range recorded in recent years [4]. Majority of the people at risk of contracting this infection live in South East Asia. The incidence in Punjab is 4.10% [5]. The aim of the study was to observe the test variations in routine Complete Blood Count (including Hemoglobin concentration, Hematocrit, Platelet count & Leucocytes count) in Dengue virus infected patients. To correlate severity of thrombocytopenia in association with NS1 Ag & IgM/IgG antibody positivity and to study seasonal variations in Dengue positive cases.

MATERIAL AND METHODS

The present study was conducted over a period of two years (March 2012 to March 2014) in department of pathology and microbiology at Adesh

Institute of Medical Sciences & Research, Bathinda after approval from ethics committee of the institute. The venous blood samples were taken at the time of admission and collected in vacationers, and were submitted to microbiology department for dengue serology testing. A rapid solid phase immuno chromatographic test for qualitative detection of antigens and differential detection of IgM & IgG antibodies to dengue virus in human serum with the help of Dengue test kit (J.Mitra & Co Pvt Ltd) was used. Dengue test kit supplied by J.Mitra & Co. is a combipack to detect both NS1 antigen and IgM/IgG antibody. Dengue NS1 antigen device contains two lines: C (control line) and T (test line). Test line is coated with antibodies, anti-dengue NS1 Ag. When a sample was added to this device, Dengue NS1 Ag if present in the sample will bind to the anti-dengue NS1 gold colloid conjugate making antigen antibody complex. This complex migrates along the membrane to the test region and forms a visible pink line at T as antibody-antigen-antibody gold colloid forms. Dengue IgM/IgG test device contains three lines : C (control line), M (IgM test line) and IgG test line. IgM test line is coated with anti-human IgM and IgG test line is coated with anti-human IgG. When a sample was added to this device, IgM and IgG antibodies in the sample react with particles coated with dengue proteins. As this sample mixture migrates along the length of test, anti Dengue IgM or IgG antibody particle complex is captured by relevant IgG/ IgM test device window causing a pale to dark red band to form at the IgG/IgM region of the test device window.

Serologically confirmed positive cases (antigen or antibody or both) were evaluated by Sysmex automated haematology analyzer XS-800i (Sysmex Corporation, KOBE, JAPAN) for haematological parameters like hemoglobin concentration, total leucocyte count (TLC), differential leucocyte count (DLC), platelet count and hematocrit. Platelet count, differential count of leucocytes and their morphology was evaluated manually on peripheral stained smear stained with leishman staining technique and further correlated with analyser readings. Results were entered in the attached Performa and were statistically analysed using Z test and SEP test (standard error of difference between the proportions).

RESULTS AND DISCUSSION

The social determinants for dengue involve population growth, the rapid urbanization process along with the slower provision of public services as potable water, sewage, and garbage collection systems, especially in suburban settings. Water storage practices and disposable water containers in suburban neighbourhoods have created a wide spectrum of breeding sites that favour mosquito densities in highly crowded areas. Dengue virus's transmission has also

increased due to the growing transport capacity and migration movements from endemic areas [6].

In the present study among 425 patients, youngest patient in the study had an age of 4 years and oldest had an age of 82 years. Average age was 38.04 years. Most of the patients were adults 417(98.12%), and only 8(1.88%) were children (< 10 years). More than 68 % of the patients were between the age 21 years to 50 years. Out of 425 patients; 258 (60.70%) were males and 167(39.30%) were females. Hence there was more number of male vis-à-vis female patients in this study. The incidence ratio between male patients to female patients was 3:2

In present study conducted on 425 serum samples, 298 (70.11%) patients were positive for NS1 only, 26(6.11%) positive for IgM only, while 7 patients had only IgG. More than one marker was detected in the remaining 94 (22.11%) samples (table I). Rashmi K.S *et al.*; [7] conducted a study on total of 4015 serum samples, out of which 1385 samples were found positive for dengue serological markers. In total, NS1 antigen was detected in 59% of cases. IgM and IgG antibodies were detected in 47.87% & 53.71 % cases respectively. Individually, NS1 Ag was detected in 32.6% cases, IgM antibodies in 5% and IgG antibodies in 15% cases, thus validating with our study.

Mean hematocrit value was 38.7% and only 15% patients had hematocrit more than 45 %. (Table II). Nazish Butt *et al.*; raised hematocrit (>40%) were found in 52(50%) of patients [8]. Balasubramaniam S *et al.*; conducted a study analyzing the usefulness of radiographic and ultrasonographic findings and area specific hematocrit cut off values in Dengue Hemorrhagic Fever (DHF) [9]. Among the DHF cases, hemo concentration (>20%) was detected in 20 cases (57.14%). The fewer number of cases showing raised hematocrit, could probably be explained as, the defined cut off for hemo concentration in the present study was > 45% as compared to >40% in the other studies.

Similar findings were observed by R.N Makroo *et al.*; [10], who conducted study on 225 serologically positive dengue cases. The mean age of the dengue patient was 27 years and the most belonged to the 21-30 year age group, which included 73 patients (32.44%). In present study, mean total leucocyte count was 5239 cells/ cu.mm and 160 patients (37.8%) had leucopenia (<4000 cells / cu.mm). 145 patients (33.95%) had relative lymphocytosis (>40%). Although there was no significant association of relative lymphocytosis; reactive lymphocytes were present in 47(11.05%) patients. Leucopenia with lymphocytosis was major findings in dengue fever. Nazish Butt *et al.*; [8] conducted a study in which out of 104 patients, 55 (52.8%) had leucopenia .The mean leucocyte count was

5200 cells/cu.mm. Curtis G.H *et al.*; in their study found relative lymphocytosis in 47.6% of patients [11].

In present study, 316 patients had a platelet count less than one lakh/ cu.mm, 64 patients had a platelet level at the low normal level ranging from 1 lakh to 1.5 lakh/ cu.mm, while 45 patients had platelet levels above 1.5 lakh/ cu.mm. Among the 316 cases of thrombocytopenia; 101 (31.96%) patients had a mild thrombocytopenia (60,000-100,000 cells/cu.mm), 143 (45.25%) had moderate thrombocytopenia (20,000-60,000 cells/cu.mm), and 72(22.78%) had severe thrombocytopenia (<than 20,000 cells/cu.mm) (Table III). The study done in Hong-Kong during 1998-2005 among 126 patients with 123 (98%) being dengue fever and 3 (2%) had dengue hemorrhagic fever, thrombocytopenia, was present in 86% of patients [12]. Narayanan et al [13] studied 59 patients, out of which 14 had a platelet count less than 50,000cells/cu.mm; 29 had platelet count between 50,000-100,000/cumm and 16 had platelet count more than one lakh/cumm. Out of 19 patients confirmed dengue in a study done by Amrit Abrol, 4 patients had thrombocytopenia less than 70,000/cumm; 1 with thrombocytopenia between 71000-81000/cumm and 2 had platelet count more than 1lakh/cumm. In addition they found thrombocytopenia was significantly associated with dengue serology positivity [14]. Nazish Butt et al [8] reported that 100% of the patients in his study, had thrombocytopenia. Lata R Patel conducted a study on 1025 samples,with 167 dengue positive cases, Platelet count was observed < 50,000 in 33(20%), between 50,000 & < 1 lac in 60(36%) and above 1 lac in 74(44%) patients [15].

Of the 425 cases, 298 (70.11%) were positive for only NS1 antigen. Considering very high specificity of NS1 it can be stated that we would have missed the diagnosis in majority of the cases, had we not included NS1 in the test panel. This supports the fact that a large number of cases would be missed if NS1 is not included in the test panel. 316 (74.11%) showed

thrombocytopenia. In 298 cases that were positive for NS1, thrombocytopenia was evident in 220 (73.82%) cases. In contrast, when only antibodies were considered for the diagnosis of dengue infection, thrombocytopenia was noted in 18 of 33 (54.44%) cases only. Association of thrombocytopenia with NS1 was found to be higher by SEP test = 9.043, Z=2.13 and p value =.0332, which is less than 0.05 suggesting significance. Further analysis of two groups NS1 only (73.82%) v/s NS1 plus IgM (89.55%) showed that thrombocytopenia was associated excellently when both NS1 and IgM were positive compared to NS1 alone (SEP =4.55 , Z=3.4, p=.0005, highly significant). Therefore, better association of platelet count with detection of antibody is consistent. The present study findings concurred with RD Kulkarni *et al.*; [16] study, concluding that thrombocytopenia correlates well when NS1 and IgM are detected simultaneously.

In a study conducted by RD Kulkarni *et al.*; of 320 serologically confirmed cases, 220 (68.8%) showed thrombocytopenia. In 130 cases NS1 positive cases, thrombocytopenia was seen in 103 (79.2%) cases. Association of thrombocytopenia with NS1 was found to be higher by SEP test = 5.01, Z=3.51 and P value <0.001, highly significant. Analysing two groups, NS1 only (73.68%) v/s NS1 plus IgM (94.12%), thrombocytopenia was associated excellently when both NS1 and IgM were positive compared to NS1 alone (SEP = 6.06, Z=3.37, P<0.001, highly significant) [17]. The seasonality of transmission of dengue with increased activity in the post – monsoon season was seen in the present study; in accordance with the reported patterns of dengue transmission and is further supported by a study conducted by Lal M *et al.*; [18]. Gupta E *et al.*; received 62 (13.6%), 279 (61.2%) and 115 (25.2%) cases respectively in September, October and November of 2005. The maximum numbers of cases were diagnosed in October, with the peak seen during the second and third week [19].

Table-1: Efficacy of various dengue specific parameters in the diagnosis of dengue infection

Parameter	Total	Percentage
NS1 only	298	70.11
NS1 + IgM	67	15.764
NS1 +IgG	13	3.05
IgM only	26	6.11
IgG only	7	1.64
IgM+IgG	14	3.29
Total	425	100

Table-2: Showing hematological parameters in 425 cases

Parameters	Haemoglobin (gm/dl)	Haematocrit (%)	WBC count (10 ³ cells/cu.mm)	Platelets count (10 ³ cells/cu.mm)
Range	7.8 to 16	25.4 to 53.2	2.1 to 3.8	10 to 193
Mean	12.03	38.7	5.288	68.901
Standard deviation	1.6054	5.5310	5.87	50.820

Table-3: Comparison of platelet counts with various permutations and combinations of dengue parameters

Dengue parameters	Total cases	Cases with platelet count less than one lakh	Percentage
NS1 only	298	219	73.48
IgM only	26	15	57.69
IgG only	7	5	71.42
NS1+IgM	67	60	89.55
NS1+IgG	13	8	61.53
IgM+IgG	14	9	64.28
Total	425	316	74.35

CONCLUSION

Basic hematological parameters can be used as indicators of dengue infections. In this study done on 425 dengue patients, the finding of thrombocytopenia was almost a universal finding validating the results of other previous studies. Presence of reactive lymphocytes was another leading finding. Inclusion of NS1 in the diagnosis of dengue increases the detection rate significantly. Thrombocytopenia correlates well when NS1 and IgM are detected simultaneously. Other associated findings included hemo concentration, leucopenia, and lymphocytosis. Using simple hematological parameters, the diagnosis of dengue could be suspected and confirmed with the help of dengue card test, utilizing immuno chromatographic test. Hence this study was undertaken to make an early diagnosis of dengue fever with the help of hematological parameters which includes hemoglobin estimation, total leucocyte count, differential leucocyte count, platelet count and leishman stained peripheral blood smear examination and dengue antigen and serological tests.

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