VOLUME-8, ISSUE-11, NOVEMBER-2019 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

DIAGNOSTIC AND PROGNOSTIC IMPORTANCE OF CBC PARAMETERS IN EARLY DENGUE INFECTION: A STUDY OF 50 CASES IN TERTIARY TEACHING MEDICAL COLLEGES

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ABSTRACT

Background: Aim of study was to determine diagnostic and prognostic importance of change in CBC parameters of NS1+ dengue patients. Certain change in TLC, DLC and HCT parameters could signify dengue infection in febrile patients. Further serial CBC findings could predict future course of disease.Dengue viruses (DV) belong to family Flaviviridae and there are four serotypes of the virus referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).It is epidemic in urban areas of Bihar..

Material and methods: Epidemiological data and hematological parameters of 50 NS1 positive early dengue fever patients were collected during epidemic period of July 2019 to October 2019 in tertiary teaching government medical colleges of Bihar, India.

Result: Out of total number of 50 patients, female was 18 and male was 32, male female ratio was 1.8:1. Minimum age was 5 years and maximum age was 64 years.TLC was either normal or decreased, predominantly normal, low TLC was in 18 patients and rest has normal TLC . .Differential showed usually normal neutrophil count or neutrophilia, 17 patients had neutrophilia and 1 had neutropenia 5 patients revealed neutropenia with lymphocytosis and monocytosis. Platelet count was usually normal range except thrombocytopenia in five patients, one had $<1,00,000/\mu L$.

Conclusion: The study results were relevant in the characterization of biological markers in the evolution of the disease and could be used as markers for early diagnosis and prognosis prediction thereby enabling health professionals in taking early help with the adaption of therapeutic conduct for specific patients.

KEYWORDS : Dengue, Dengue Fever, Dengue Shock Syndrome, NS1, Thrombocytopenia,Neutrophilia

INTRODUCTION:

Dengue viruses (DV) belong to family Flaviviridae and there are four serotypes of the virus referred to as DENV-1, DENV-2, DENV-3 and DENV-[4][1]. It is found mainly in areas of the tropic and sub-tropics. It is a positive stranded encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated (M) protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins (1). It is transmitted mainly by Aedes aegypti mosquito and also by Aedes albopictus . NS1 has got diagnostic importance. All four serotypes are antigenically similar but infection with one serotype confers lifelong immunity against that serotype only. Infection with other serotypes culminates in more serious infection. All four serotypes can cause the full spectrum of disease from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) (3) . The WHO 2009 classification divides dengue fever into two groups: uncomplicated and severe (1), though the 1997 WHO classification is still widely used. Aim of study was to determine diagnostic and prognostic importance of change in complete blood count (CBC) parameters of NS1+ dengue patients. Certain change in total leukocyte count (TLC), DLC (differential leukocyte count) and hematocrit(HCT) parameters could signify dengue infection in febrile patients. Further serial CBC findings could predict future course of disease (4).The 1997 classification divided dengue into undifferentiated fever, dengue fever (DF), and dengue haemorrhagic fever (DHF). Four main characteristic manifestations of dengue illness are (i) continuous high fever lasting 2-7 days; (ii) haemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; (iii) thrombocytopenia (platelet count <1,00,000/ul). According to the ADE hypothesis, secondary dengue virus infections are risk factors for DHF/dengue shock syndrome. There are thus two reasons to find a simple test to distinguish between primary and secondary infection with early serum samples. The first reason is to be able to carry out an epidemiological study to check whether the incidence of severe cases of dengue is significantly higher among secondary infections than among primary infections. The second reason is to know the immunological status of patients to allow clinicians to take it into account in the progression of the disease until the ADE hypothesis has been confirmed or disproved. DV antibody reactivity patterns serve as useful tools for classifying patients as having primary or secondary DV infection. Detection of DENV IgM in the absence of DV IgG (i.e., an IgM-positive/IgG negative reactivity pattern) is a clear indicator of primary DV infection. Similarly, an IgM+ IgG+ pattern combined with low IgG avidity accurately identifies primary DV infection .An IgM+ IgG+ reactivity pattern with high IgG avidity is an accurate marker of secondary infection among patients whose serum samples are collected within a month of symptom onset (5, 6,7). 1.1 Pathogenesis could be understood by cellular and tissue tropism. Cellular tropism: three types of cells are involved: (1) immune cells (2) endothelial cells (3) cells of liver and other organs (8,9,10). In immune system, primarily monocytes/macrophages are infected, responsible for cytokine release syndrome (11). The mechanisms that have been considered to cause DHF include antibody-dependent enhancement (ADE) T cell response and a shift from Type 1 T helper (Th-1) to type 2 T helper (Th-2) responses(9,10,11). Th-1 cells produce interferon-gamma, interleukin (IL)-2, and tumour necrosis factor (TNF)-beta, which activate macrophages and are responsible for cell-

mediated immunity and phagocyte-dependent protective responses. type 2 Th (Th-2) cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses(12,13). The combined effect of all of these is cytokine release syndrome resulting in movement of body fluids in extravascular space. Various cytokines have been implicated in the immuno-pathogenesis of DF/DHF. It has been suggested that in dengue a Th1 response is linked to recovery from infection while a Th2 type response leads to severe pathology and exacerbation of the disease. Vasculopathy is characterized by attachment of major nonstructural protein NS-1 to membrane of endothelial cells leading to cell retraction and increased vascular permeability, with clinical manifestation of hemorrhage and ultimately shock in few cases. The microvasculature of lung and gut is preferentially affected, that is radiologically manifested with pleural effusion and ascites. In liver, hepatocytes and kupffer cells are affected, producing hypoxia and release of AST and ALT, however severe hepatic damage is not established till now. So coagulopathy is not due to factor deficiency. Secondary dengue infection with different serotype is more serious due to phenomenon of antibody enhancement of disease. The production of excessive antibody against NS1 antigen by memory cells is one of the probable cause of DHF/DSS .Therefore antibody is not protective. Also autoantibody effect is seen, due to cross-reactivity with other tissue of human being. DENV-2 inhibits in vitro megakaryopoiesis and induces apoptotic cell death in a subpopulation of early megakaryocytic progenitors which may contribute to thrombocytopenia in dengue disease. In another study it was shown that DV-2 may directly interact with and activate platelets and thus may be responsible for thrombocytopenia. Still the exact cascade of mechanisms involved in dengue disease pathogenesis remains unexplained and lot more needs to be done.

METHOD AND MATERIAL:

Epidemiological data and hematological parameters of 50 NS1 positive early dengue fever patients were collected during epidemic period of July 2019 to October 2019 in tertiary teaching government medical colleges of Bihar, India. Age, gender and CBC findings were noted and tabulated. In Few atypical cases peripheral blood smears (PBS) were made to see atypical lymphoid cells and to know RBC morphology in anaemic patients. Manual platelet counts were done in each and every case.

Table: 1. Hematological Parameters in early Dengue fever (Ns1+) and IgM(-) & IgG(-)

CASE NO.	Age (years)	sex	TLC	N	L	RBC	HB	HCT	PLT
1	34	F	2.8	48	48	4.12	12.2	37.3	160
2	8	F	7.8	66	24	4.64	12.2	37.5	210
3	30	M	6.7	45	48	4.44	12.1	35.6	300
4	35	F	5.8	86	10	4.78	12.4	40.2	155
5	35	F	3.4	73	22	4.25	11.5	36.3	180
6	42	M	4.8	84	8	3.82	12.3	37.7	165
7	38	M	3.0	48	42	4.38	13.0	40.5	160
8	50	F	4.5	88	5	3.93	11.9	37.4	160
9	45	M	6.2	75	16	5.04	14.7	44.6	240
10	60	F	5.0	70	22	3.55	10.08	34.7	180
11	41	M	5.6	76	16	3.75	13.2	39.0	160
12	29	F	3.1	60	30	3.99	11.0	34.9	180
13	50	F	3.6	67	29	4.81	11.5	37.4	160
14	27	F	5.8	85	9	3.48	8.3	28.0	190
15	15	M	2.8	58	35	4.46	13.0	44.0	155
16	8	M	4.1	61	34	4.74	12.5	38.7	170
17	10	M	8.1	81	12	4.69	12.3	38.2	180
18	17	M	1.6	48	35	5.09	15.3	46.1	155
19	24	M	7.1	88	6	6.40	14.6	45.3	160
20	19	F	5.6	85	12	5.17	16.1	47.8	170
25	21	M	1.6	77	20	4.40	12.4	38.2	155
26	5	M	6.20	84	12	4.20	11.2	34.5	200
27	59	F	3.4	77	20	5.22	14.0	44.2	155
28	23	M	3.5	85	11	5.03	13.5	41.4	150
29	39	M	5.6	69	20	5.25	15.1	46.3	80
30	55	M	5.0	68	30	4.24	12.4	37.9	150
31	33	M	2.4	55	36	5.23	15.9	48.2	120
32	22	F	7.5	84	10	4.30	12.3	36.9	185
33	52	F	6.3	82	12	4.07	7.6	24.5	180
34	64	F	3.4	39	50	4.73	12.2	37.3	120
35	50	M	3.7	77	17	4.06	10.9	34.7	100
36	15	M	4.7	64	30	5.64	16.0	48.6	160
37	15	M	5.80	76	16	5.01	14.6	43.8	180
38	51	M	4.3	78	15	4.75	13.5	41.1	150
39	31	M	2.9	63	33	5.29	14.2	43.9	150
40	7	M	6.3	89	07	4.71	12.1	38.7	150
41	50	F	5.4	85	10	4.38	12.3	39.1	170
42	20	F	5.3	85	11	4.43	10.3	33.0	240
43	44	F	3.9	32	60	4.88	14.4	44.4	174
44	12	F	4.2	82	12	4.70	13.0	39.3	175
45	15	M	4.5	84	10	4.36	10.7	34.4	200
46	35	M	6.6	84	09	4.65	13.1	41.4	140
47	48	M	5.2	75	20	3.69	12.9	36.2	160
48	17	M	2.80	72	22	4.12	14.1	43.3	150
49	39	M	3.4	77	17	4.10	13.8	40.9	130
50	21	F	3.0	78	17	3.62	11.0	34.6	150

RESULT:

Out of total number of 50 patients , female was 18 and male was 32 ,male female ratio was 1.8:1. Minimum age was 5 years and maximum age was 64 years.TLC was either normal or decreased, predominantly normal, low TLC was in 18 patients and rest has normal TLC . Differential showed usually normal neutrophil count or neutrophilia, 17 patients had neutrophilia and 1 had neutropenia 5 patients revealed neutropenia with lymphocytosis and monocytosis. Platelet count was usually normal range except thrombocytopenia in five patients , one had<1,00,000/μL. However in follow up cases, progressive leucopoenia and hemoconcentration (raised HB/RBC count/ HCT) was usual findings(Table 1).However seven patients revealed low hemoglobin with microcytic hypochromic picture mostly in female.PBS revealed atypical large lymphoid cells in many cases that usually reflected as monocytosis in CBC(Figure 2).

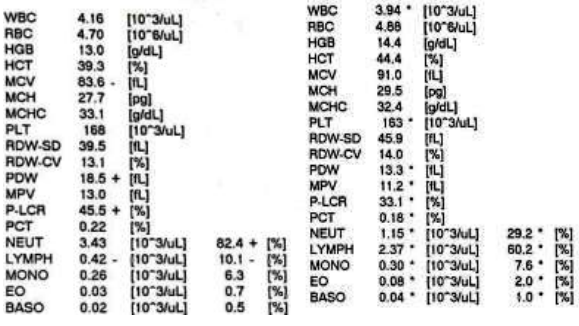
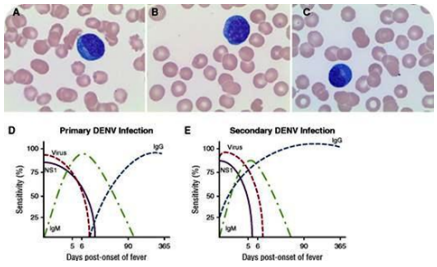


Figure: 1. Recovery of platelet count in Dengue infection



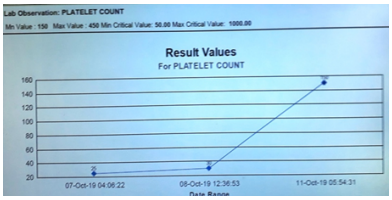


Figure: 2. Reactive lymphocytosis in Dengue and pattern of antibody response

DISCUSSION

Dengue fever is an infectious disease which is difficult to distinguish clinically from other viral fever prevalent in our region (1). However it does not present with cold and cough. This study aimed at analyzing clinical and epidemiological data and CBC parameters in order to identify biomarkers that are predictive of severity. In our study, early dengue fever cases with NS1 positive were prevalent possibly because the out patients were referred for evaluation of febrile cases presented with malaise, headace, severe bodyace or joint pain, nausea, vomiting and abdominal fullness. The frequency of dengue fever in the study was higher in the group aged 10 years old or over. There was a predominance of men in this study; in most published studies, there is no significant difference in the proportions by gender (15, 16).

Disease was more severe in individuals aged 50 years and older with a more pronounced and persistently low TLC, neutropenia, lymphocytosis and hemoconcentration, manifested by high RBC count, hemoglobin and hematocrit, HCT must be kept <45%. Platelet count is important severity marker but till count is not <20,000/ul, naturally recovery is expected (Figure 1).Thus message is to keep careful watch in CBC parameters change especially in old group patients (25, 26).

CONCLUSION:

Dengue fever evolves with laboratory alterations starting on day one with NS1 positivity, subsequent biochemical and hematological changes become evident on the 3rd day and becoming most evident on the 5th day .Early change in CBC like neutrophilia with normal or decreased TLC, neutropenia with low TLC, high RBC count, high hemoglobin and high hematocrit (PCV) are hematological markers of dengue infection. Subsequent CBC findings and peripheral blood smears have got prognostic significance, especially 20% or more rise in PCV and less than <20% platelet count are alarming sign. They could indicate impending dengue hemorrhagic fever/ dengue shock syndrome .The study results are relevant in the characterization of biological markers in the evolution of the disease and can be used as markers for the most severe forms thereby enabling health professionals in taking early help with the adaption of therapeutic conduct for specific patients. It could be useful for making policy regarding early diagnosis and management of dengue fever in state like Bihar on basis of changes in CBC parameters.

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