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Research Article

Correlation Between Hematological and Serological Parameters in Dengue Fever Patients

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Abstract

According to the World Health Organisation, dengue is endemic in more than 100 countries. there are an estimated 390 million or more cases of dengue annually worldwide, and 40% of the world population. Understanding the pathophysiology of dengue virus infection along with an early diagnosis of dengue fever with the aid of laboratory parameters and clinical evaluation helps in the management of dengue fever. The objective of this study was to analyze the hematological parameters which will help in the early diagnosis of dengue syndromes. Most patients are clinically asymptomatic. However severe dengue fever may cause increased vascular permeability with vascular leakage, hemoconcentration, hypovolemic shock, and bleeding manifestations due to thrombocytopenia. The parameters studied were hemoglobin concentration, total leucocyte count, differential leucocyte count, platelet count, and Leishman-stained peripheral smear examination. In our study, out of 100 dengue-positive patients proven by the ELISA method; the common laboratory finding was thrombocytopenia (97%). Leucopenia was found in only 13% of patients and 18% of patients had relative lymphocytosis. Of 100 patients 81% of patients showed the presence of reactive lymphocytes. Also noted was a raised hematocrit in 21% of patients. The correlation of dengue IgM antibody with hematological parameters was studied. The severity of thrombocytopenia depends inversely on the titre of antibody level in the patient.

Keywords: Dengue Fever; Dengue Hemorrhagic Fever; Dengue Shock Syndrome; Hemoconcentration; Thrombocytopenia; Dengue IgM Antibody

Introduction

According to the World Health Organization (WHO), dengue is considered one of the most common arthropod-born infections; affecting about 390 million infections annually and 40% of the world's population [1]. Dengue is a single-stranded RNA virus that belongs to the Flaviviridae family. It is an endemic virus in tropical countries. Dengue virus has serotypes (DENV1, DENV2, DENV3, and DENV4). The main vector is Aedes aegypti and most infections are asymptomatic or mild [2]. In some patients, dengue fever (DF) can develop into life-threatening fatal disease forms, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [3].

Pathophysiology of dengue hemorrhagic fever

Virulence of the virus, pre-existing dengue antibodies, immune system disturbances, lipid changes, and genetic susceptibility of the host are factors that have been reported to correlate with the development of DHF. However, the exact causes and mechanisms that trigger DHF are controversial [4]. Dengue virus (DENV) infection causes a characteristic pathology in humans,

accompanied by vascular dysregulation. In some patients with dengue hemorrhagic fever (DHF), vascular pathology may become severe, causing extensive microvascular permeability and leakage of plasma into tissues and organs, resulting in hemoconcentration and hypovolemic shock [5].

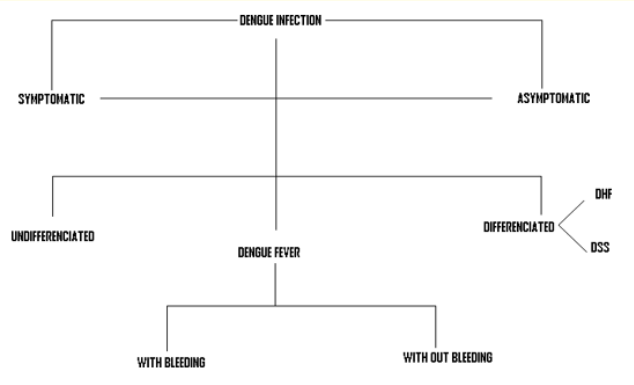

Figure 1: Manifestation of Dengue fever.

Immunopathogenesis

Immune reactions such as the overproduction of cytokines and the formation of autoantibodies against platelets and endothelial cells occur after infection with the dengue virus. Molecular mimicry between platelets or endothelial cells with NS-1 antigen or dengue virus prM would explain the cross-reactivity of antiNS1 or anti-prM antibody with host cells and participate in the invasion of platelets and endothelial cells during disease development. Strong immune activation in dengue leads to monocyte and macrophage activation by cytokines, which leads to phagocytosis of autoantibody-coated platelets and promotes the development of DHF/DSS. Coagulopathy and plasma leakage are the most important pathological changes in the clinical manifestations, mortality, and morbidity of DHF. Plasma leakage develops rapidly or slowly and stops completely and predictably after 2-8 hours, possibly due to functional change rather than structural vascular damage and inflammation [4].

Symptoms and manifestations

Symptoms of Dengue fever include [6,7].

- Fever
- Headache

Cytokines	Dengue fever	DHF
IL-1	markedly↑	↑
IL-2	↑	Markedly ↑
IL-4	↑	Markedly ↑
IL-8	↓	Markedly ↑
IL-10	↓	Markedly ↑
IL-12	Markedly ↑	↓
IL-13	↑	Markedly ↑
IL-18	↑	Markedly ↑
INTERFERON- α	Markedly ↑	↑
TNF- α	Markedly ↑	Markedly ↑
Transforming Growth factor	↓	Markedly ↑
Cytotoxic factor	↑	Markedly ↑

Table 1: Immunopathogenesis of dengue fever.

- Myalgia
- Joint pains
- Abdominal pain
- Vomiting
- Dizziness
- Skin rash
- Flushing

The severity of DHF is classified into 4 classes.

- **Grade 1:** Fever accompanied by nonspecific symptoms, the only hemorrhagic manifestation is a positive tourniquet test
- **Grade 2:** Spontaneous bleeding in addition to manifestations of Grade 1 patients, usually in the skin
- **Grade 3:** Circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mm of Hg or less), or hypotension with the presence of cold clammy skin and restlessness.
- **Grade 4:** Profound shock with, undetectable blood pressure and pulse.

Early diagnosis helps to prevent mortality. Simple hematological parameters, Helps in the diagnosis of dengue. The aim of the study was to make an early diagnosis of dengue fever by hematological

parameters such as hemoglobin estimation, total leucocyte count, differential leucocyte count, platelet count, and Leishman-stained peripheral blood smear examination. The correlation of Dengue antibodies to laboratory parameters may help in the advanced effective treatment of dengue fever. Understanding the pathophysiology of dengue fever due to NS1 antigen helps the development of NS1-targeted vaccines and may provide different opportunities to combat dengue fever.

Materials and Methods

The study was conducted in St: John's Medical College Hospital Bangalore, on 100 IgM-positive dengue patients proven by the ELISA method of antibody estimation.

Age-gender distribution

Among the 100 patients; 21 were children (less than 12 years), and the ages ranged from 2 to 60 years with a mean of 26.9. years.

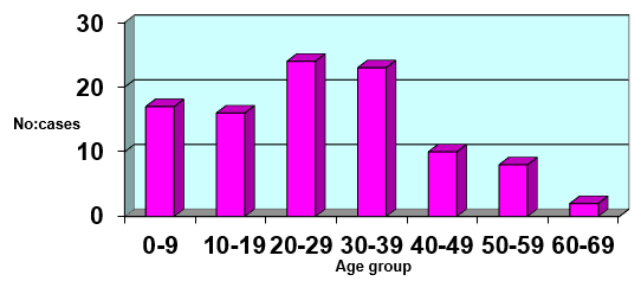

Figure 2: Shows the distribution of cases according to different age groups.

Most of the patients were adults (79%).

Figure 3: Shows children adult ratio of affected dengue.

Figure 4: Shows male female ratio of affected dengue.

Methods used

The EDTA sample of these patients was examined for

- Hemoglobin count* (Sodium lauryl sulphate method)
- Total leucocyte count* (Flow cytometry)
- Differential leucocyte count* (Hydrodynamic focusing)
- Platelet count* (Electrical impedence).

Tests 1 to 4 were done by using the automated instrument "SYSMEX XT 2000i".

- Examination of Leishman-stained peripheral smear examination was done to verify the above results and to note any morphological abnormalities. On Leishman-stained smear reactive lymphocytes were graded from 0+ to 4+).
- Enzyme immunoassay for dengue IgM antibody estimate.

Results

Hemoglobin

The hemoglobin levels among these patients ranged from 6.7-17.5 g/dl with a mean of 12.6 g/dl.24% had a hemoglobin level of more than 15 g/dl. Out of this 23 were males and 1 was female. Two of the cases with elevated hemoglobin were children.

Hematocrit

21% of patients had hematocrit more than 45%. The hematocrit value ranged from 21.6 to 55.2% with a mean hematocrit value of 38.3%.

Figure 5: Blood Smear closely packed RBCs (due to hemoconcentration).

Total leucocyte count

Total leucocyte count ranges from 2500-32000 cells/cu.mm; with a mean of 7823.2 cells/cu.mm. Only 13% had leucopenia (<4000 cells/cu.mm) which ranged from 2500-4000/cu.mm.

Differential Leucocyte count

In this study, 18 patients had relative lymphocytosis (>45%). The reactive lymphocytes were graded from 0+ to 4+ from Leishman-stained smears. The reactive lymphocytes were graded from 0+ to 4+ from Leishman-stained smears. The plasmacytoid appearance of reactive lymphocytes was significant.

Figure 6: Grading of reactive lymphocytes.

Although relative lymphocytosis was recorded only in 18% of cases, reactive lymphocytes were found in 81% of patients. Most of the patients (n = 39) showed grade 1+ reactive change (up to 20% of lymphocytes with reactive morphological changes), 26% and 16% showed grade 2+ and 3+ reactive change respectively. No reactive changes in the lymphocytes were seen in 19% of cases.

Reactive lymphocytes (gradings)	1+	2+	3+	4+
(No of patients)	39	26	16	0

Table 2: Grading of reactive lymphocytes.

Figure 7: Blood smear showing reactive lymphocytes.

Platelet count

The severity of thrombocytopenia was graded as mild–moderate–severe according to the criteria in the following table.

Mild	Moderate	Severe
60,000-1,00000 cells/cu.mm	20,000-60,000 cells/cu.mm	< than 20,000 cells/cu.mm

Table 3: Grading of thrombocytopenia.

The range of platelet count was 9000-1.3 lakhs with a mean platelet count of 52,840 cells/cu.mm. 40 (41.2%) patients had a mild thrombocytopenia; 41 (42.2%) had moderate thrombocytopenia, and 16(16.4%) had severe thrombocytopenia. In this study, 3 patients had a platelet level at the low normal level ranging from 1.2 lakhs to 1.3 lakhs.

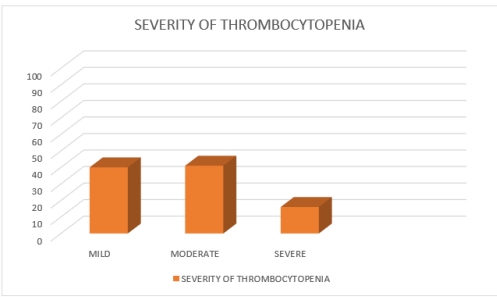

Figure 8: Percentage Severity of thrombocytopenia.

Statistics was done using SPSS VERSION 17.0.

Correlation of IgM antibody tire with various parameters

The important laboratory findings seen in the present study are given below.

Correlations					
			TITRE	Hb	Total count
Spearman's rho	TITRE	Correlation Coefficient	1.000	.125	.121
		Sig. (2-tailed)	.	.215	.230
		N	100	100	100
	Hemoglobin	Correlation Coefficient	.125	1.000	-.140
		Sig. (2-tailed)	.215	.	.164
		N	100	100	100
	Total count	Correlation Coefficient	.121	-.140	1.000
		Sig. (2-tailed)	.230	.164	.
		N	100	100	100
	Lymphocyte	Correlation Coefficient	-.118	.026	-.228*
		Sig. (2-tailed)	.243	.801	.023
		N	100	100	100
	Eosinophil	Correlation Coefficient	.094	.203	-.014
		Sig. (2-tailed)	.393	.064	.902
		N	84	84	84
	Hematocrit	Correlation Coefficient	.132	.937**	-.133
		Sig. (2-tailed)	.192	.000	.186
		N	100	100	100
	Platelets	Correlation Coefficient	-.359**	-.125	-.028
		Sig. (2-tailed)	.000	.214	.784
		N	100	100	100
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed). (p value < 0.05 is significant)					

Table 4: Correlation of IgM antibody tire with various parameters.

In this study for only platelet count, the 'p' value was less than < 0.0001 which was significant. The platelet count had a negative correlation with the IgM titre (r = -0.359); which means that when there is an increased IgM titre there is a low platelet count i.e.; severity of thrombocytopenia depends inversely on the titre level of antibody in the patient. The 'p' value was not found to be significant for other parameters including hemoglobin, hematocrit, total WBC count, etc.

Summary of laboratory findings

Parameters:	Hemoglobin (g/dl)	Hematocrit (%)	WBC count (Cells/cu.mm)	Platelet (Cells/cu.mm)	IgM titre
Range	6.7-17.5	21.6-55.2	2500-32000	9000-1.3 lakhs	1.0-5.6
Mean	12.6	38.3	7823.2	52840	2.02
Std. Deviation	2.5674	7.4770	8056.598	27957.298	.74339

Table 5: Showing summary of laboratory findings.

Observed Significant parameters in 100 patients

Increased Hb(>15 g/dl)	Raised hematocrit (>45%)	Leucopenia (<4000 cells/ cu.mm)	Relative lymphocytosis (>45%)	Reactive lymphocytes	Thrombocytopenia
24%	21%	13%	18%	81%	97%

Table 6: Significant parameters observed.

ELISA for dengue antibody

The cases included in the study were all positive for IgM, indicative of acute infection and 19 patients showed IgG positivity also.

Discussion

In this study out of 100 dengue-positive patients diagnosed with ELISA; a common laboratory finding was thrombocytopenia (97%). Leukopenia was found in only 13% of patients, and relative lymphocytosis was present in 18% of patients. Of 100 patients, 81% had reactive lymphocytes. An increased hematocrit was also found in 21% of patients. The correlation of dengue IgM antibody with hematological parameters was investigated. The severity of thrombocytopenia is inversely related to the patient's antibody concentration. The platelet count had a negative correlation with the IgM titre (r = -0.359); which means that when there is an increased IgM titre there is a low platelet count i.e.; severity of thrombocytopenia depends inversely on the titre level of antibody in the patient. The 'p' value was not found to be significant for other parameters including hemoglobin, hematocrit, total WBC count, etc.

In a similar study by Imran Qadir., *et al*. among 152 dengue patients, 45 had bicytopenia (leukopenia and thrombocytopenia (29.6%) [8]. In a study by Surendra Nath (2018) on serologically confirmed dengue patients, the most common hematological abnormality was thrombocytopenia (92%), followed by leukopenia (68%). An increased hematocrit was observed in 23 patients. Peripheral smear showed atypical lymphocytes in 9 patients, and typical findings were plasmacytoid lymphocytes [9]. Reactive lymphocytes were present in 81% of patients in our study. Clarice., *et al*. (2019) highlighted the AL (atypical lymphocytosis) rate of clinical signs of severe dengue is significantly higher than that of dengue without warning signs and AL rate at presentation may predict severe DI, and future larger longitudinal studies should have established that AL rate at admission predicts disease complications [10]. Patil., *et al*. (2020) described in their study that dengue IgM can be detected within 3-5 days in 50% of people after the onset of symptoms, 80% on the 5th day, and 99% on the 10th day. After 2-3 months, IgM antibodies may not be detectable. IgM is not specific; they can cross-react with other flaviviruses and also with rheumatoid factors [11,12]. In our study comparison of IgM antibody titre was correlated with other hematological parameters, however, only platelet count had an inverse correlation to the titre of the Dengue IgM antibody. Thrombocytopenia is a consistent laboratory feature in dengue fever [13,14]. Thrombocytopenia was a consistent laboratory feature in our study 97% of patients had thrombocytopenia ranging from mild-moderate-severe. Increased Vascular permeability leads to plasma leakage leading to hemoconcentration in dengue patients. An increased hematocrit was also found only in 21% of patients.

Limitations of the Study

Dengue ELISA kits are sometimes associated with cross-reactivity with chikungunya virus. Whenever possible, molecular technology should be used whenever the number of co-infections is higher than expected to exclude cross-reactivity [15,16]. Cross-reactivity of chikungunya antibodies in Dengue ELISA is a possibility. Simultaneous diagnosis of chikungunya and dengue infection is needed to diagnose coinfection which was not performed in our study.

Conclusion

Basic hematological parameters can be used as indicators of dengue infections.

In this study done on 100 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. In addition, we graded the presence of reactive lymphocytes from 1+ to 4+ depending on the number. We found 65% of cases had up to 40% of lymphocytes in the peripheral blood showing reactive changes. The recording of reactive morphological changes in lymphocytes was not analyzed by the previous studies.
- Other associated findings included hemoconcentration, leucopenia, and lymphocytosis.
- Platelet count had an inverse correlation to the titre of the Dengue IgM antibody.

With the aid of laboratory parameters including hematological profile, early diagnosis of dengue fever is possible which helps in early treatment. Until now, treatment against dengue has been mostly supportive, with no authorized therapeutic drugs. Understanding the role of therapeutic approaches in NS1 pathogenesis helps the development of NS1-targeted vaccines and may provide different opportunities to combat dengue fever.

Acknowledgement

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Conflict of Interest

There is no conflict of interest.

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