



HAEMATOLOGICAL PROFILE IN DENGUE FEVER

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ABSTRACT **Background:** 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.

Objectives: To analyze the hematological parameters which will help, in the early diagnosis of dengue syndromes. The parameters studied included hemoglobin, total leucocyte count, differential leucocyte count, platelet count and Leishman stained peripheral smear examination.

Materials & Methods: Study was conducted in Katuri Medical College, Guntur, Andhra Pradesh on 100 IgM positive dengue patients proven by ELISA method of antibody estimation.

Results: Out of 100 dengue positive patients proven by ELISA method; the common laboratory findings were thrombocytopenia (in 97% patients). Leucopenia was found in 13% of patients and 18% of patients had relative lymphocytosis. Reactive lymphocytes were seen in 81% of patients. Also noted was a raised hematocrit, seen in 21% of patients.

Conclusion: In the present study, thrombocytopenia and reactive lymphocytes were the commonest associated findings. The other laboratory parameters like hemoconcentration, leucopenia and lymphocytosis are also noted. They can be used as indicators for early diagnosis of dengue fever.

KEYWORDS :

INTRODUCTION

Dengue is endemic in more than 100 countries. There is an estimated of 50 million or more cases of dengue annually worldwide with 4,00,000 cases of dengue hemorrhagic fever⁽¹⁾. The frequency of dengue and its more severe complications dengue hemorrhagic fever and dengue shock syndrome has been dramatically increased since 1980. 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⁽²⁾. The disease warrants early diagnosis for effective treatment to prevent mortality.

Using simple hematological parameters, the diagnosis of dengue could be suspected. Hence this study was undertaken to make an early diagnosis of dengue fever by hematological parameters which include hemoglobin estimation, total leucocyte count, differential leucocyte count, platelet count and Leishman stained peripheral blood smear examination.

OBJECTIVE

To analyze the hematological parameters which will help in the early diagnosis of dengue syndromes.

MATERIALS REQUIRED

Study was conducted in Katuri Medical College, Guntur, Andhra Pradesh, on 100 IgM positive dengue patients proven by ELISA method of antibody estimation.

SAMPLE

EDTA blood sample

METHODS USED

- The EDTA sample of these patients were examined for
- 1) Hemoglobin count* (Sodium lauryl sulphate method)
 - 2) Total leucocyte count (Flow cytometry)
 - 3) Differential leucocyte count (Hydrodynamic focusing)
 - 4) Platelet count (Electrical impedance)
- Tests 1 to 4 were done by using automated instrument "SYSMEX XT 2000"
- 5) 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+.
 - 6) Enzyme immuno assay for dengue IgM antibody estimate

RESULTS

One hundred patients diagnosed as dengue based on ELISA IgM positivity were included in the study.

Age Distribution:

Among the 100 patients; 21 were children (<than 12 years), and the

ages ranged from 2 to 60 years with a mean of 26.9 years.

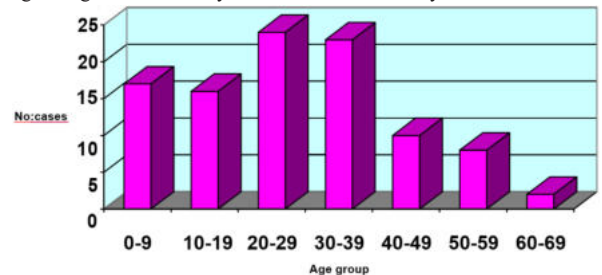


Fig 1: Showing The Distribution Of Cases According To Different Age Groups

Most of the patients were adults (79%).

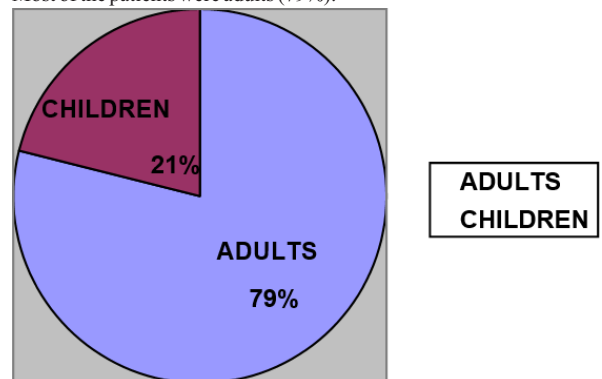


Fig:2 Adult: Children Ratio Of Affected Dengue Patients

Gender Distribution

In present study 71 were males and 29 were females.

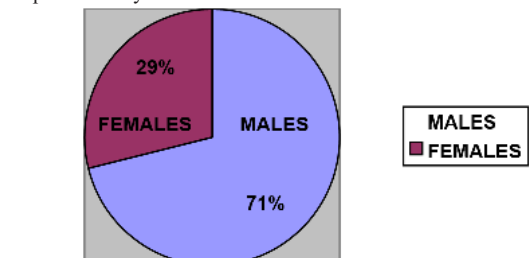


Fig 3: Showing M: F Ratio In 100 Dengue Patients

HEMATOLOGICAL FINDINGS

Hemoglobin

The hemoglobin levels among these patients ranged from 6.7-17.5g/dl with a mean of 12.6g/dl.24% had a hemoglobin level of more than 15 g/dl. Out of this 23 were males and 1 female. 2 of the cases with elevated Hemoglobin were children.

Hematocrit

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

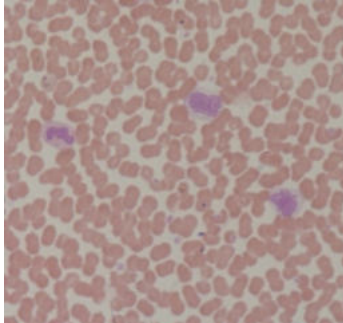


Fig 4: Blood Smear closely packed RBCs (due to hemoconcentration) (Leishman stain 1000X)

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/cmm.

Differential Leucocyte Count

In this study 18 patients had relative lymphocytosis (>45%).

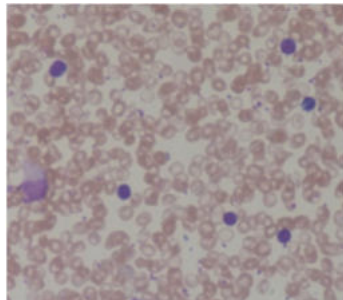


Fig 5: Blood Smear Showing Lymphocytosis (leishman Stain 400X)

The reactive lymphocytes were graded from 0+ to 4+ from leishman stained smears.

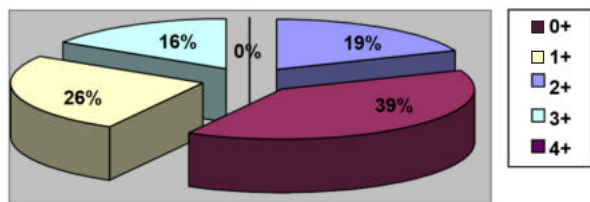


Fig 6: Showing Grading Of Reactive Lymphocytes From Leishman Stained Blood Smears.

Although relative lymphocytosis was recorded only in 18% cases, reactive lymphocytes were found in 81% 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% cases.

Table 1: Grading Of Reactive Lymphocytes

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

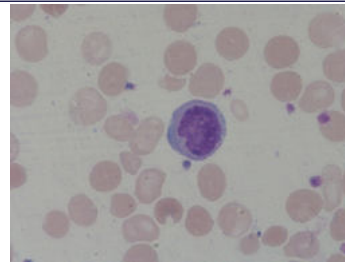


Fig 7: Blood Smear Showing Reactive Lymphocytes (leishman Stain 1000X)

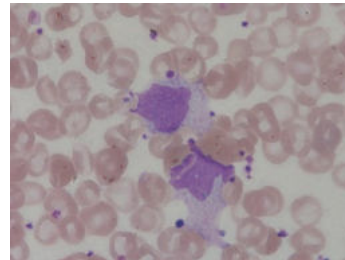


Fig 8: Blood Smear Showing Reactive Lymphocytes With Monocytoid Appearance (Leishman Stain 1000X)

The severity of *thrombocytopenia* was graded as mild-moderate-severe according to the criteria in Table

Table 2: Grading Of Thrombocytopenia

MILD	MODERATE	SEVERE
60,000-1,00000 cells/cu.mm	20,000-60,000 cells/cu.mm	< than 20,000 cells/cu.mm

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.

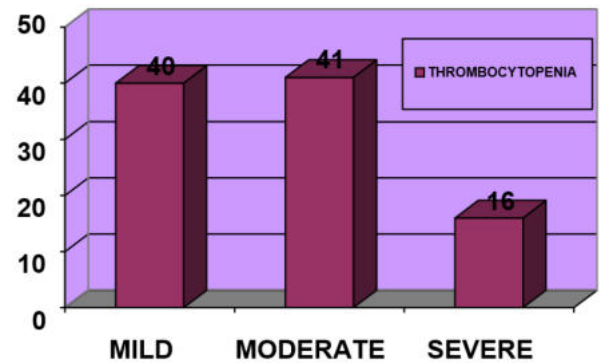


Fig 9: Distribution Of Cases According To The Degree Of Thrombocytopenia

In this study, 3 patients had a platelet level at the low normal level ranging from 1.2 lakhs to 1.3 lakhs.

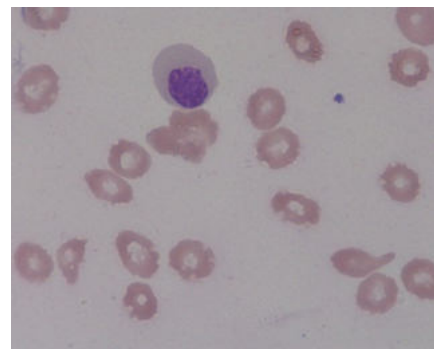


Fig 10: Blood Smear Showing Thrombocytopenia With nRBC (Leishman stain 1000X)

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

Table 3: Summary Of Laboratory Findings

Parameter s:	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	9000-1.3lakhs	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 4: Correlation Of IGM Antibody Tite With Various Parameters

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)

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.

Table 5: Observed Significant Parameters In 100 Patients

Increased Hb(>15g/dl)	Raised hemato crit (>45%)	Leucopen ia (<4000 cells/cu.m m)	Relative lymphoc ytosis (>45%)	Reactive lymphoc ytes	Thrombocy openia
24%	21%	13%	18%	81%	97%

ELISA For Antibodies

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

DISCUSSION:

Dengue virus is a member of the Flavi virus family, which includes yellow fever and various other mosquito and tick borne encephalitis. It presents as four antigenically distinct serotypes (DENV-1, -2, -3 and -4). Dengue fever has become a major health issue affecting tropical and sub-tropical regions around the world – especially urban and peri-urban areas⁽³⁾, Dengue has a worldwide distribution and remains a

health problem or potential threat in areas infested with *Aedes aegypti* mosquito. In India, the first outbreak of dengue fever was recorded in 1812, virus was isolated in 1945 and many epidemics have been reported since then. The severe fatal forms of disease, i.e. ,dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) though common in South East Asia since early 1940's were reported in India for the first time from Calcutta in 1963. Since then epidemics of DSS/DHF have been reported from various states of India. Similar epidemics have been reported from other South East Asian countries, namely, China, Thailand and Indonesia⁽⁴⁾. In India, dengue epidemics have been reported in many parts of the country. The incidence was reported to be high among children _ 8 years of age, and some infants presented with severe forms of dengue. Due to lack of awareness of dengue in general, the present surveillance system in India is unlikely to generate reliable dengue epidemiological database information, which is essential for better clinical assessment and management⁽⁵⁾. Infections with any types of dengue virus causes a spectrum of illness ranging from no symptoms or mild fever to severe and fatal hemorrhage and shock developing largely on the patient's age and immunological condition⁽⁶⁾. Most dengue virus infections in children have minimal or no symptoms and cannot be easily distinguished clinically from other viral infections. Classical dengue fever (DF) is an acute illness characterized by fever, retroorbital headache, severe myalgias, and occasionally a rash which lasts from 5 to 7 days. In a small percentage of dengue infections, a more severe form of disease, known as dengue hemorrhagic fever (DHF). DHF is characterized by acute fever associated with a hemorrhagic diathesis and to develop shock (dengue shock syndrome), which distinguish it from DF. Abnormal hemostasis and plasma leakage are the two pathophysiological hallmarks of DHF, which are manifested clinically as thrombocytopenia and hemoconcentration (pleural effusion)⁽⁷⁾.

AGE & SEX:

Dengue affects humans of all age groups worldwide. In some parts of the world, it is mainly a pediatric health problem. Dengue in infancy has also been reported from India and Sri Lanka⁽⁸⁾. A study done by Gurdeep S.D & et al in 81 cases of dengue fever 53 were males(65.4%) and 28(34.5%) were females. Children between 10-15 years were most commonly affected (59%) (38) In the present study among 100 patients 71 (71%) were males and 29 (29%) were females. Most of the patients were adults (79%), and only 21 (21%) were children (<than 12 years).

HEMATOLOGICAL PARAMETERS

Hemoglobin & Hematocrit

In a study done by Gurdeep S.D & et al; the mean hematocrit value was 35.5⁽⁸⁾. In another study done by Nazish Butt & et al, raised hematocrit (>40%) were found in 52(50%) of patients⁽³⁾. Baruah. J et al in 44 patients 40 (90.9%) had a hemoglobin value > 14.5 g/dl⁽⁹⁾. In the present study the mean hemoglobin value was 12.6g/dl; 24 patients had a hemoglobin level of more than 15 g/dl. The mean hematocrit value was 38.3 % and only 21% patients had hematocrit more than 45 %.The fewer number of cases showing raised hematocrit, could probably be explained as , the defined cut off for hemoconcentration in the present study was > 45% as compared to >40% in the previous study.

Total And Differential WBC Counts

Leucopenia with lymphocytosis was major findings in dengue fever. In a study done by Ole Wichmann et al, 53.2% patients had leucopenia⁽¹⁰⁾. Nazish Butt & et al, in their series found that, out of 104 patients 55 (52.8%) had leucopenia. The mean leucocyte count was 5200 cells/cu.mm⁽³⁾. Curtis G.H and et al in their study found relative lymphocytosis in 47.6% of patients⁽¹¹⁾. In our study mean total leucocyte count was 7823.2 cells/ cu.mm and 13% had leucopenia (<4000 cells / cu.mm). 18 patients had relative lymphocytosis (>45%). Although there was no significant association of relative lymphocytosis, reactive lymphocytes was present in 81 (81%) of patients. In addition, we graded the presence of reactive lymphocytes from 1+ to 4+ depending on the number. We found 65% cases had upto 40% of lymphocytes in the peripheral blood showing reactive changes, while 16% cases showed upto 60% of the lymphocytes with reactive changes. The recording of reactive morphological changes in lymphocytes was not analyzed by the previous studies.

Platelet Count:

Thrombocytopenia and dysfunctional platelets remains a central hallmark of dengue fever, surprisingly little is known about the

interaction of dengue virus with platelets⁽¹²⁾. A 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⁽¹³⁾. A study done by Narayanan et al in 59 patients 14 had a platelet count less than 50,000cells/cu.mm;29 had platelet count between 50,000-100,000 and 16 had platelet count more than one lakh⁽¹⁴⁾. Out of 19 patients confirmed dengue in a study done by Amrit Abrol, 4 patients had thrombocytopenia less than 70,000; 1 with thrombocytopenia between 71000-81000 and 2 had platelet count more than 1lakh. In addition they found thrombocytopenia was significantly associated with dengue serology positivity⁽⁶⁾. In the Nazish Butt & et al study 100% of the patients had thrombocytopenia⁽³⁾. In our study 97 patients had a platelet count < than one lakh & 3 patients had a platelet level at the low normal level ranging from 1.2 lakh to 1.3 lakh. Of these patients 40 (41.2 %) patients had a mild thrombocytopenia; (60,000-100,000cells/cu.mm), 41 (42.2%) had moderate thrombocytopenia (20,000-60,000 cells/cu.mm), and 16(16.4%) had severe thrombocytopenia(<than 20,000 cells/cu.mm).

The present study findings concurred with the previous studies and we found that *thrombocytopenia* was the most commonly associated finding and there was a negative correlation of thrombocytopenia with IgM titre.

TREATMENT AND MANAGEMENT OF DENGUE FEVER

Early and effective replacement of losses with plasma, plasma expander and/or fluid and electrolyte solution results in a favourable outcome in most cases. With adequate fluid administration, DSS is rapidly reversible. Rapid replacement will usually prevent clinical DIC. Prognosis depends upon early recognition of shock, based on careful monitoring. It is not necessary to hospitalize all suspected cases of DHF, since shock may develop in only about one – third of patients. The constant finding that a drop in the platelet count usually precedes the rise in hematocrit is of great diagnostic and prognostic value. In order to be able to recognize the early signs of shock, and thus to take preventive action, parents should be advised to bring the patient back for repeat platelet and hemoconcentration determinations. A watch should also be kept for any signs of clinical deterioration or warning of signs and shock, e.g.: restlessness, and/or lethargy, acute abdominal pain, cold extremities, skin congestion, or oliguria, usually on or after third day of illness.⁽¹⁵⁾

SUMMARY

Dengue is endemic in more than 100 countries. There is an estimated of 50 million or more cases of dengue annually worldwide, with 4,00,000 cases of dengue hemorrhagic fever.

The objective of this study was to analyze the hematological parameters which will help in the early diagnosis of dengue syndromes. 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 ELISA method; the common laboratory finding was thrombocytopenia (97%). Leucopenia was found in only in 13% of patients and 18% of patients had relative lymphocytosis. Of 100 patients 81% of patients showed presence of reactive lymphocytes. Also noted was a raised hematocrit in 21% of patients.

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% 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.

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