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STUDY OF HEMATOLOGICAL PARAMETERS IN DENGUE PATIENTS

Pathology

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ABSTRACT

Background: Dengue fever caused by the dengue virus and transmitted by Aedes aegypti and Aedes albopictus mosquitoes, is often misdiagnosed as other tropical fevers. Hematological parameters play important role in characterization of progression of the disease as well as early management. Analysis of these gives enough clues so as to diagnose dengue severity and in their initial stages and thus facilitates early treatment and observation of dengue cases. This would minimize morbidity and mortality arising out of serious complications of dengue fever. **Methods:** Diagnosis was confirmed using reverse transcription polymerase chain reaction and NS1 antigen/IgM antibody ELISA. Hematological data (hemoglobin, hematocrit, platelets, WBC count) were collected over the period as patients were diagnosed as dengue-positive. **Results:** Hematocrit, platelet count, and total WBC count declined during illness, indicating disease severity. Differential counts revealed reactive lymphocytosis and neutropenia as the disease progressed. **Conclusion :** Hematological parameters are key in tracking disease progression and guiding early management of dengue. Their analysis helps identify severity in the initial stages, enabling timely treatment and reducing the risk of serious complications, morbidity, and mortality.

KEYWORDS

Dengue Fever, Hematocrit, Platelet Count , Lymphocytosis

INTROCUTION

Dengue is a mosquito transmitted disease caused by dengue virus, often self-limiting and frequently asymptomatic . But in India, It has been very complex disease and has substantially changes over many decades in terms of prevalent strains, affected geographical locations and severity of disease.[1] It is often goes misdiagnosed or unrecognized as other fever causing tropical diseases. [2]

There's no unique remedy for dengue, but the well timed diagnosis of dengue cases, identity of warning signs for intense dengue, and appropriate scientific management are key elements of care to prevent the development to extreme dengue and deaths.[3]

Dengue is diagnosed by different antigen and antibody detection methods such as virus specific serological test, molecular detection and virus isolation. Three most common markers used are NS 1, Ig M for acute infection and Ig G for previous infection. NS – 1 antigen can be detected from the first 0 to 9 days of symptoms onset, and Ig M is detected 4 to 5 days after symptom onset , and production may continue approximately for more then 3 months. Ig G levels can be detected throughout the life, from 10 to 14 days of postinfection.

Active dengue detection via Immunochromatography , while user-friendly, easy to use and with rapid turnover time, has low sensitivity and low specificity, as well as higher cross-reactivity leading to more false positive results. Thus, Hematological parameters can be beneficial as a supportive test for dengue diagnosis in addition to rapid dengue test via Immunochromatography methods.

Therefore, this study attempts to provide an in-depth analysis of different hematological parameters with detection of serological marker NS1 and /or IgM of acute dengue infection. Thus, Incorporation of hematological parameters may act as a supportive parameters for proper management to prevent the life-threatening consequences of dengue.[4]

This study aims to analyze the alteration of hematological parameters in adult patients diagnosed with dengue at tertiary care hospital.

METHOD :

The present Observation study was conducted in Department of Pathology, Tertiary care hospital, which includes 370 adult patients (age >18 years) with dengue fever confirmed by ELISA (NS-1 antigen/IgM antibody Positive) admitted in medicine wards. Case papers of all the patients were studied and their hematological data were collected.

Hematological Analyzer : Hematological data were analyzed using this fully automated hematology analyzer, which works on principle of electronic impedance and optical light scatter for cell counting and differential analysis.

RESULT AND OBSERVATION :

In the present study , out of 370 patients 265(71.62%) patients were Male and 105(28.38 %) were female. Male female ratio is 2.5:1, suggest that males are more affected then females.

Table 1 : Gender distribution among Dengue patients

Gender	No. Of people	Percentage (%)
Male	265	71.62
Female	105	28.38
Total	370	100

Majority of patients 340 (92%) were presented with fever, 213 (56%) were having headache, 159 (43%) patients were having bodyache, rashes and retro-orbital pain were observed in 38 (10%) and 31 (8 %) respectively. 4% of patients were having petechia, <1 % patients were having bleeding manifestations such as bleeding gums ,epistaxis and melena. Very few patients were having ascites, shock and breathlessness.

Table 2 : Showing frequences of symptoms/signs shown by dengue patients

Symptoms/signs	Shown by no. of patients	Percentage (%)
Fever	340	92
Rashes	38	10
Headache	213	56
Bodyache	159	43
Retro-orbital pain	31	8
Petechia	15	4
Bleeding gums	3	0.8
Epistaxis	5	0.5
Melena	5	0.5
Ascites	2	0.2
Shock	2	0.2
Altered sensorium	1	0.002
Breathlessness	1	0.002

In our study, Most of the cases 342 (92%) were having Dengue Fever. While 24 (6%) cases had Dengue Hemorrhagic Fever and 4 cases (1%) were of Dengue Shock Syndrome.

Table 3 : Clinical spectrum of dengue positive cases		
Diagnosis	**No. of cases**	**Percentages(%)**
Dengue Fever	342	92
Dengue Hemorrhagic Fever	24	6
Dengue Shock Syndrome	4	1
Total	370	100

1) Hematocrit :

On Day 1, 59.35 % of patients of DF had normal HCT , 120 patients had low HCT and 19 were had high HCT. Among DHF patients, majority of patients had normal HCT, few had low and few had high HCT. In DSS patients, 3 had normal HCT and 1 patient had high HCT.

On Day 3, 73 and 8 patients of DF had low and high HCT respectively. Majority of DHF patients were having normal HCT, only few had low HCT. One patient of DSS had high HCT and rest had normal HCT. On Day 5, All patients of DSS had normal HCT.

Out of 370 patients, 234 patients (220 DF +13 DHF + 1 DSS) had low Hemoglobin<12 gm/dL on Day 1. On Day 3, 239 patients (223 DF + 14 DHF + 2 DSS) had low hemoglobin and On day 7, All DF and few of DHF patients were recovered, One of DSS patient died, so few patients had (0 DF + 5 DHF + 1 DSS) low hemoglobin.

Table 4 : Distribution of Haematocrit (HCT) according to grading of the dengue infection				
		DF	DHF	DSS
Day 1	Low (<36)	120	4	0
	Normal(36-46)	203	16	3
	High (>46)	19	4	1
	Total	342	24	4
Day 3	Low (<36)	73	3	0
	Normal(36-46)	261	20	3
	High (>46)	8	1	1
	Total	342	24	4
Day 5	Low (<36)	0	0	0
	Normal(36-46)	0	0	3
	High (>46)	0	0	0
	Total	0	0	3

Table 5 : Derivation of Mean Hct and P value					
		Mean Difference	P-	95% Confidence Interval	
HCT		(I-J)	Value	Lower Bound	Upper Bound
D1	D3	.17854	1.000	-1.0417	1.3987
	D5	33.64399*	.000	31.7188	35.5691
D3	D1	-.17854	1.000	-1.3987	1.0417
	D5	33.46545*	.000	31.5403	35.3906
D5	D1	-33.64399*	.000	-35.5691	-31.7188
	D3	-33.46545*	.000	-35.3906	-31.5403

The hematocrit (HCT) levels show significant changes across days. Between Day 1 and Day 3, the mean difference is 0.17854 with a p-value of 1.000 (95% CI: -1.0417 to 1.3987), indicating no significant change. However, comparing Day 1 to Day 5, the mean difference is 33.64399 with a p-value of 0.000 (95% CI: 31.7188 to 35.5691), showing a significant decrease. Similarly, from Day 3 to Day 5, the mean difference is 33.46545 with a p-value of 0.000 (95% CI: 31.5403 to 35.3906). These results highlight a significant drop in HCT levels by Day 5.

Hematocrit (HCT) levels over time show significant variations. On Day 1, the mean HCT is 36.90 (±6.78), which slightly decreases to 36.72 (±5.72) on Day 3. However, by Day 5, there is a notable drop to 3.26 (±10.76). This dramatic decrease on Day 5 indicates a significant change in HCT levels over the observed period.

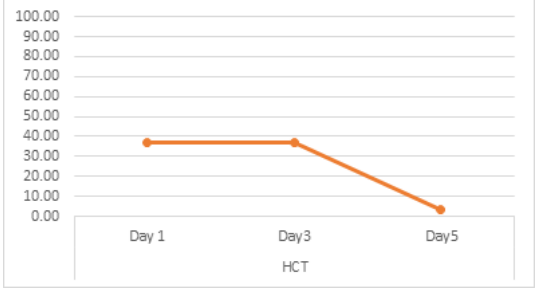

Chart 3 : Comparison of mean HCT for day 1,3,and 5

2. Platelet Count

In our study, 265 patients of Dengue Fever showed thrombocytopenia on Day 1 , few of them were recovered and 67 had thrombocytopenia. All of DHF and DSS patients had thrombocytopenia on Day 1,3 and 5.

Table 6 : Platelets distribution according to grading of dengue infection				
		DF	DHF	DSS
Day 1	Normal (>1,50,000)	77	0	0
	Low (<1,50,000)	265	24	4
		342	**24**	**4**
Day 3	Normal (>1,50,000)	275	0	0
	Low (<1,50,000)	67	24	4
		342	**24**	**4**
Day 5	Normal (>1,50,000)	0	0	0
	Low (<1,50,000)	0	09	3
		0	09	3

Out of 370 patients, 342 patients who had Dengue Fever majority 304(82%) were having thrombocytopenia (platelet count < 1,50,000/ cumm). 159 patients of Dengue fever showed moderate thrombocytopenia, few had sever thrombocytopenia. Among 24 patients of DHF, 11 and 11 patients respectively had moderate and sever thrombocytopenia. Whereas Dengue shock syndrome presented by 24 patients and all of them had sever thrombocytopenia.

Table 7 : Derivation of Mean platelet and P value						
		Mean	Std.	P-	95% Confidence Interval	
PLT		Difference (I-J)	Error	Value	Lower Bound	Upper Bound
D1	D3	-81.359	3.864	0.000	-90.630	-72.089
	D5	-20.755	18.780	0.808	-65.815	24.304
D3	D1	81.359	3.864	0.000	72.089	90.630
	D5	60.604	18.780	0.004	15.544	105.664
D5	D1	20.755	18.780	0.808	-24.304	65.815
	D3	-60.604	18.780	0.004	-105.664	-15.544

Platelet (PLT) levels show significant changes between days. From Day 1 to Day 3, the mean difference is -81.359 with a p-value of 0.000 (95% CI: -90.630 to -72.089), indicating a significant increase. Comparing Day 1 to Day 5, the mean difference is -20.755 with a p-value of 0.808 (95% CI: -65.815 to 24.304), showing no significant change. From Day 3 to Day 5, the mean difference is 60.604 with a p-value of 0.004 (95% CI: 15.544 to 105.664), indicating a significant decrease. These results highlight significant variations in PLT levels from Day 1 to Day 3 and Day 3 to Day 5.

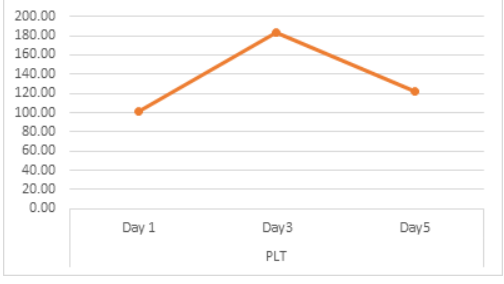

Chart 2 : Comparison of mean Platelet for day 1,3,and 5

Table 8: Thrombocytopenia on the basis of severity of Dengue			
Dengue Fever	DHF	**DSS**	**Thrombocytopenia**
148	3	0	Mild (1,49 to 1,01)
159	11	0	Moderate(1 to 50,000)
13	10	4	Sever(< 50,000)
320	24	4	Total

3) Total Wbc Count :

Leucopenia (< 40,000/cumm) was observed in 43.85 % of DF , 66 % of DHF, 75 % of DSS patients on Day 1, following days patients with leucopenia were reduced. Total WBC count(TC) levels over time show slight fluctuations. On Day 1, the mean TC is 5.32 (±3.20). This slightly decreases to 5.30 (±2.74) on Day 3. By Day 5, the mean TC rises to 6.13 (±1.55).

Table 9: Patients with leucopenia according to grading of Dengue infection			
Leucopenia	DF	DHF	DSS
Day 1	150/342	16/24	3
Day 2	75/342	10/24	3
Day 3	0	0	2

Along with leucopenia, lymphocytosis(>40 lymphocytes/cumm) is also observed in Dengue patients. 19 % of DF, 87 % of DHF and 50 % of DSS showed lymphocytosis on Day 1. Following Days few of DHF and DSS patients had showed lymphocytosis.

Table 10 : Grading of Dengue according with Lymphocytosis

Lymphocytosis observed in following cases			
	DF	DHF	DSS
Day 1	67	21	2
Day 3	35	3	2
Day 5	0	0	1

DISCUSSION

Most patients presented with anemia, thrombocytopenia and leukopenia on initial stage of illness. These early hematological changes are indicative of the initial viral impact on bone marrow and immune system dysregulation. There was a significant reduction in platelet counts along with some patients with increased platelet count suggestive of recovery. Few patients showed further leukopenia with a notable lymphocytosis (Neutropenia). This period marks the intensification of bone marrow suppression and immune response to the virus. On advancement of the disease ,the nadir of thrombocytopenia was typically observed. The fluctuations in hematological parameters over these days underscore the importance of regular monitoring to manage potential complications such as bleeding.

The dynamic changes observed in these parameters offer significant insights into the progression of dengue infection and have important implications for clinical management and prognosis. Thus, no single parameter has been identified to effectively assess the severity of the disease.[5]

The study comprised 370 adult patients who were hospitalized in tertiary care hospitals with dengue virus infection. Out of the total of 370 patients, 265 were male and 105 were female.

Supriya Karmakar et al.[6]Out of 210 cases , 115 (54.7 %) were males and 95 (45.2%) were females, similar with our study.

Timely recognition of warning indicators such as abdominal discomfort, ongoing vomiting, and mucosal hemorrhage is essential for effective management and the prevention of complications. [7-8]

1. Hematocrit (HCT)

In the present study, Hematocrit (HCT) levels vary significantly over time. It is because, hemoconcentration usually occurs in patients with dengue shock syndrome and was not much altered in classical dengue fever.

Hematocrit (HCT) levels showed significant variation over time. While no significant change occurred (p = 1.000). The sharp drop suggests a significant reduction in HCT levels over the observation period, likely due to the absence of hemoconcentration, which is more common in dengue shock syndrome than classical dengue fever.

These findings align with previous studies, including those by **Supriya Karmakar et al's** [6] and **Prof. Sanjay Mehrotra et al.**[9], which reported varying HCT levels depending on disease severity.

The mean hemoglobin levels decreased over a period as disease progress. Overall 63.24 % of total population had anemia (hemoglobin <12 gm/dL) on initiation of disease ,in which 220 cases were (64 %) of DF, and 223 cases were of (65 %) DHF . 54 % patients of DHF had anemia on progression of disease. Few cases of DSS also had anemia .

2. Platelet (PLT) levels

In the current study, Platelet (PLT) significantly reduced as the disease progress. 79.18 % of total population had thrombocytopenia, in which majority of cases 265 were diagnosed as DF . Few cases of DHF and all cases of DSS showed moderate to severe thrombocytopenia.

These results align with previous studies, such as those by **Supriya Karmakar et al's**[6], **Prof. Sanjay Mehrotra et al.**[9], **JuthatipChaloemwong et al.**[10] and **Lilian Karem Flores-Mendoza et al**,[11] which reported trends of thrombocytopenia peaking during early to mid stages of illness, especially in dengue patients. Potential causes of thrombocytopenia may include (I) suppression of bone marrow progenitor cells by the dengue virus, (II) heightened apoptosis resulting in platelet destruction, (III) complement-mediated lysis, (IV) the formation of anti-platelet antibodies, and (V) disseminated intravascular coagulation resulting in platelet consumption. The median total platelet count was notably greater in the non-survivor group, indicating that platelet count may not serve as a reliable predictor of mortality, a finding corroborated by several studies. [12-15]

3. Total Leucocyte Counts (TC)

Leucopenia was observed in 45.67% of dengue-positive patients, notably in DHF and DSS cases. Atypical lymphocytes were seen in some cases. Total leukocyte counts (TC) remained stable across days, with no significant differences (p = 1.000). Lymphocytosis was more common in severe cases (87% in DHF, 50% in DSS), while neutrophilia appeared during recovery. These results align with prior studies by **Supriya Karmakar et al's**[6], **Juthatip Chaloemwong et al**[10], **Lilian Karem Flores-Mendoza et al.**[11] and others, showing disease severity correlates with WBC trends.

Leucopenia were found in 45.67 % of total dengue positive patients, observed significantly in DHF and DSS patients during onset of dengue. As it reached to its peak only DHF and DSS patients had leucopenia.

Presence of atypical lymphocytes were also observed in few cases.

Atypical lymphocytes are commonly observed in various viral infections, such as infectious mononucleosis, herpes, rubella, influenza, and viral hepatitis. While their precise role remains uncertain, these cells incorporate elevated levels of [3H] thymidine into deoxyribonucleic acid and resemble lymphocytes that undergo blast transformation when stimulated by mitogens like phytohemagglutinin. Therefore, it is possible that atypical lymphocytes signify a reaction to either non-specific viral stimulation or specific viral antigens, resulting from recognition and subsequent transformation.([16-17] The mean differences in total Counts (TC) levels between days are not statistically significant.

CONCLUSION

Early treatment, including adequate hydration and monitoring of vital signs and laboratory parameters such as hemoglobin, hematocrit, platelet count and total leukocyte count can prevent progression to severe forms of the disease, such as dengue hemorrahagic fever or dengue shock syndrome. Regular follow up and supportive care are essential to manage symptoms and reduce the risk of mortality.

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