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Unfamiliar hematological parameters predictive of dengue

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ABSTRACT

Dengue is an endemic viral disease in India with frequent outbreaks. The clinical manifestations are protean and can vary from persistent fever for few days and minor hematological changes to severe forms which include life threatening complication of dengue shock syndrome. The present study was aimed to identify hematological markers which can aid reflex dengue serology testing for timely diagnosis. Hematological profile of a total of 53 patients with serological diagnosis of dengue was studied. Thrombocytopenia and leucopenia were seen in 32% and 26% of cases respectively. Raised hematocrit was noticed in only two cases. Trend of increase in mean of absolute lymphocyte count was observed on correlation of hematologcial values with duration of fever. Similarly, a dip in mean for absolute neutrophil count in patients with fever of 4 – 6 days duration was noticed. Hematology analyzer XN-550 showed "atypical lymphocyte" as the most common leucocyte flag amongst study group. The present study showed that classical hematological abnormalities of dengue such as thrombocytopenia and raised hematocrit in dengue are present in low frequency. The other findings such as rising absolute lymphocyte counts with duration of fever, low neutrophil counts in fever of 4 to 6 days duration, reactive lymphocytes and peripheral smear, abnormal flags of "atypical lymphocyte" and "blasts" shall be referred for dengue serology testing.

1. Introduction

Dengue, a systemic viral infection is transmitted by Aedes mosquito. The clinical manifestations of the disease vary from fever to fatal complications such as shock syndrome.[1,2] Despite substantial vector control measures, the worldwide incidence is on the rise. Climate changes, mass migration to urban areas and rapid urbanization are postulated to contribute to this increasing trend.[3] The rising incidence in resource poor and marginalized countries has compelled to label it as a neglected tropical disease.[4] Other febrile arboviral infections and endemic diseases such as typhoid, malaria, leptospirosis, viral hepatitis present with similar clinical features. Serology and hemogram are the common preliminary investigations ordered in management of dengue. Several viral infections can present with similar laboratory abnormalities.[5,6] The absence of effective vaccine, specific treatment and failed vector control measures reinforce the need to explore the role of hematological parameters in laboratory management of dengue viral infection.[1,7] Hence, the present study aims to identify hematological makers associated with higher probability of dengue, thereby facilitating disease surveillance. In the present study, we attempt to characterize hematological derangement in various stages of disease by correlating with serological test results. Along with hematological results, various flags outlined by automated Hematology analyser have been described herein.

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2. Material and Methods

The present descriptive study was conducted at a tertiary care centre in coastal Karnataka after obtaining ethical clearance from institutional ethical committee. All consecutive cases of laboratory confirmed dengue on serology over a period of six months were included in the study. Results of dengue serology performed using Dengue Check combo kit from non-anticoagulated blood sample were collected. Serology results were interpreted following adherence to standard recommendation as shown in Table 1.[8] Demographic details such as age, sex, hospital identifiers and duration of symptoms were collected. Any patients with incomplete or unavailable laboratory data were excluded from the study. Complete blood counts generated on first drawn EDTA sample collected at the time of consultation or as part of routine investigations were recorded. Any leucocyte flags outlined by Hematology analyzer "Sysmex XN 550" were recorded. The data was statistically analyzed in EXCEL.

3. Results

Out of total 157 serological positive cases, 53 had complete blood counts recorded. 36 among the study group were males. The study group also included 5 children. NS1 positivity seen in 20 (38%) was the most common serological result. Total leucoyte count ranged from 1,520 cells to 33,080 cells/μL with mean of 5,500 cells/μL. Mean hemoglobin and platelet count for the study population were 13.9 gm% and 135 X 10^3 /μL respectively. Thrombocytopenia, lymphocytosis and leucopenia were seen in 17/53 (32%); 10/53 (19%) and 14/53 (26%) cases respectively (Table 1). Increased hematocrit and thrombocytopenia together was observed in two patients. The hematological parameters were correlated with duration of fever (Table 2). Cases were grouped into three based on duration of fever as 1-3 days, 4 – 6 days and 7 – 9 days. Mean for Absolute lymphocyte counts showed distinctive increasing trend from 985.4/μL in 1-3 days of fever to 1732.5/μL in those with fever of 7 -9 days duration. A dip in mean for absolute neutrophil count to 2124 /μL was observed in group with 4 – 6 days duration of fever. Reactive lymphocytes of varying morphology were observed on peripheral smear in 20 out of 53 cases. (Figure 1). A total of 25 blood samples were flagged for "atypical lymphocyte" and 16 samples for "blast" by Sysmex XN-550 (Table 3).

4. Discussion

Worldwide about 2.5 billion people are at risk of dengue as reported by WHO. About 50 million people are affected annually resulting in approximately 25000 deaths.[1,4,9] In endemic areas, large numbers of febrile patients suspected with dengue are referred to hospital which overburdens the health care system. Consequently effective triage strategies

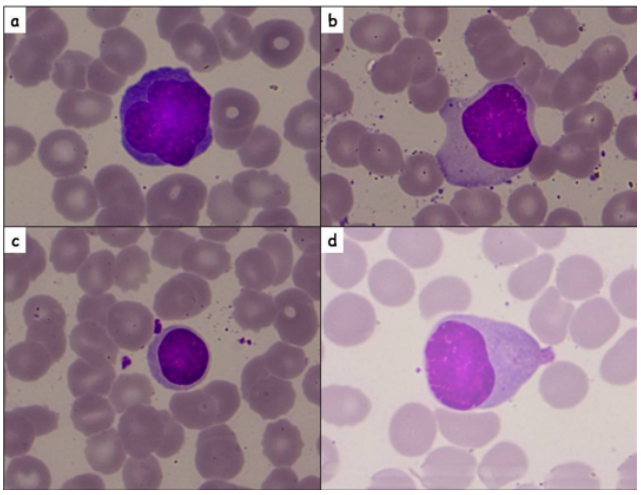

Figure 1: Reactive lymphocyte on peripheral smear:**(a)**: Downey type I [Leishman stain, X1000]; **(b):** Downey type II [Leishman stain, X1000]; **(c):** Downey type III [Leishman stain, X1000]; **(d):** Plasmacytoid lymphocyte [Leishman stain, X1000].

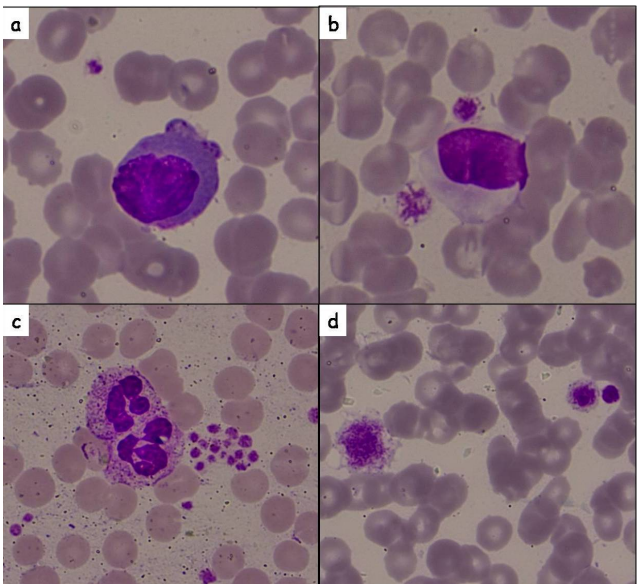

Figure 2: Platelet morphology on peripheral smear: **(a),(b)** & **(c):** Platelet-leucocyte aggregrates [Leishman stain, X1000]; **(d):** Macrothrombocyte [Leishman stain, X1000]

shall ensure optimal utilization of the limited resources available in tertiary health care centers. As complete blood count is a common preliminary investigation performed, it is prudent to explore complete blood count as a possible referral tool for dengue serology tests.

A newer consensus WHO classification classifies dengue as non-severe and severe disease. Non-severe is further classified as with/without warning signs. This new classification is based on level of severity which dictates the clinical management. Warning signs also expedite early

Table 1: Correlation of serological results with hematological abnormalities

Serology Test Result	Interpretation	Distribution	Hematology Abnormality	Frequency of Hematological abnormality
NS1 positive	Early Primary Case	20	Thrombocytopenia	06 (30%)
			Leucopenia	08 (40%)
			Lymphocytosis	04 (20%)
NS1& IgM positive	Late Primary case	05	Thrombocytopenia	04 (80%)
			Leucopenia	03 (60%)
			Lymphocytosis	02 (40%)
IgM positive	Late Primary Case	19	Thrombocytopenia	05 (26%)
			Leucopenia	00 (0%)
			Lymphocytosis	01 (5%)
IgG positive	Old dengue case	09	Thrombocytopenia	02 (22%)
			Leucopenia	03(33%)
			Lymphocytosis	03 (33%)

Footnote: Values are presented as frequency and proportion(%). Thrombocytopenia: platelet <1 lakh/μL; Leucopenia: white cells <4,000/μL; Lymphocytosis: absolute lymphocyte count >4,400/μL.

Table 2: Trends of hematological parameter in dengue with duration of illness.

Duration of fever, days	Number of patients	Mean Total white cell (10^3 /μL)	Mean Absolute lymphocyte count (/μL)	Mean Absolute neutrophil count (/μL)	Mean Platelet count (10^3 /μL)
1-3	13	4.78	985.4	2962.9	133.3
4-6	13	4.41	1532	2124.3	140.5
7-9	27	6.72	1732.5	3638.2	134.8

Footnote: All laboratory results were acquired from the first drawn sample at initial consultation.

Table 3: Common flags outlined by automated analyzer

Duration of fever, days	Number of patients	"Atypical Lymphocytes" Flag	"Blast" Flag
1-3	13	5(38%)	3(23%)
4-6	13	6 (46%)	4 (30%)
7-9	27	14 (52%)	9 (33%)

detection of clinical severity which needs critical care. Amongst the new criteria required for defining dengue, thrombocytopenia and raised hematocrit are underscored as warning signs.[2,4,9]

The haematological derangements that are seen classically described in dengue are thrombocytopenia, leukopenia and hemoconcentration.[9] However, in the present study each of these abnormalities were seen in smaller proportion of cases (Table 1). The observations in the present study are discussed herein.

In a study by L'Azou et al to assess efficacy of chimeric live attenuated dengue vaccine, dengue infection was found to be common in children aged 5 - 12 years.[10] Young age and female sex have been identified as risk factors for severe disease.[7] One out of five children with dengue in the present study had both thrombocytopenia and raised hematocrit.

Seventeen of 53 cases in the present study had thrombocytopenia with platelet counts less than 1,00,000/μL. Thrombocytopenia in dengue often necessitates treatment with platelet transfusion. The mechanisms of platelet destruction include IgG aggregates and platelet-leukocyte aggregates (Figure 2). Additionally, megakaryopoiesis is suppressed in viral infection.[6] On histology myelosuppression can be demonstrated as early as fourth day of fever, the marrow cellular reverts to normal by seventh to tenth day of fever.[5] There is also evidence for platelet dysfunction induced by dengue virus through a surface receptor, Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-Integrin. This binding stimulates apoptosis of platelets.[7]

Out of 17 patients with thrombocytopenia in the present study, four had fever of less than three days duration. Thrombocytopenia within first three days of illness is one of the validated early predictors in severe dengue disease.[11] Potts et al in a longitudinal observational study of 1,311 patients with dengue showed that not all patients with dengue hemorrhagic fever have thrombocytopenia.[12] In a study on laboratory profile of 154 dengue patients, thrombocytopenia was observed from 4th day in classical dengue fever, but was seen from the onset of disease

among severe forms.[13] Similarly, Kalayanarooj et al. in a prospective study of 60 patients with acute viral infection noted lower platelet counts at first laboratory entries in those who developed dengue hemorrhagic fever.[14] A prospective observational study was designed to investigate predictive value of daily platelet counts in determining risk for dengue shock syndrome. Among 2,301 children with dengue, lower platelet count (80000 – 150000/μL) observed between day 3 and 8 of illness was associated with progression to shock syndrome than those with higher platelet count (100000 – 180000/μL).[15] Therefore, it is surmised that absolute platelet counts in early febrile phase are independent risk factor for progression to severe disease.[2,15]

Only two cases with raised hematocrit were encountered in this study. Both the cases exhibited thrombocytopenia too. Kalyanarooj et al noted elevated hematocrit in only 12 of 25 cases with dengue hemorrhagic fever. Absence of elevated hematocrit was attributed to infrequent hematocrit estimation, increased oral intake or intravenous infusions.[14] Intravenous fluid resuscitation remains the only choice for managing shock in dengue. Hence, early and timely recognition of severe dengue is necessary. Raised hematocrit can be utilized as a laboratory tool for detecting cases with high risk of plasma leakage and shock.[4] NS1 binds to heparin sulphate, a component of endothelial glycocalyx resulting in its disruption.[7] A systemic leak syndrome characterized by hypoproteinemia, hemoconcentration, pleural effusion or acute shock follows endothelial dysfunction. Thus, NS1 antigenemia seen during acute phase is associated with severe disease.[2,7] Infected platelets elaborate interleukin-1ß which also promotes vascular permeability.[6] Antibodies produced against non-structural protein 1 (NS1) are shown to react with platelets and endothelial cells. These interactions are regarded to result in raised hematocrit.[5]

In the present study, leucopenia was seen in 12 out of 36 cases with illness of duration more than 3 days, but only two out of 13 with fever of less than three days duration had leucopenia. In a study by Azin et al, leucocytosis was seen in first few days of fever followed by leucopenia appearing later.[13] Leucopenia is attributed to myelo-suppression by dengue virus.[14]

The mean of absolute lymphocyte count steadily increased with duration of fever in the present study. The lowest mean for absolute lymphocyte count 985.4 cells/μL was observed in patients with less than 3 days fever (Table 2). Similar observation of decreased lymphocytes at onset of fever and lymphocytosis as disease progressed by 4th to 6th day was made by Raimunda et al.[13]

In a study of 1,921 patients with laboratory confirmed dengue, the lowest levels of absolute neutrophil counts were recorded on fourth to fifth day of illness with median duration of one day. Lower mean absolute neutrophil count in the present study among patients with fourth to sixth day of fever is in agreement with results of Thein et al (Table – 2).Virus induced Myelo-suppression is attributed for such transient neutropenia.[16] In a study of children with dengue, leucopenia and decreased neutrophil count was shown to have substantial discriminatory value to differentiate dengue from other febrile illness.[14]

Secondary infection with heterotypic serotype is epidemiological risk factor for severe disease. Antibody dependent enhancement, wherein pre-existing non-protective antibodies meditate viral replication in macrophages results in cytokine cascade, complement activation, endothelial dysfunction and platelet destruction, altogether precipitating plasma leakage and fatal hemorrhagic manifestation.[7,17] No cases of secondary dengue infection were encountered in the present study. We presume serotyping by RT-PCR would have revealed the dominant or co-existing serotype prevailing in the region.

Reactive lymphocytes classical of viral infection displayed characteristic varied morphology on peripheral smear evaluation. Downey type I has an indented nucleus and scant basophilic cytoplasm (Fig 1a). Downey type II has abundant pale cytoplasm often abutting adjacent red cells (Fig 1b). Type III has conspicuous nucleoli (Fig 1c) and plasmacytoid lymphocyte has polar nucleus, mesh like chromatin and basophilic cytoplasm (Fig 1d).[18] The dense basophilia of cytoplasm is attributed to accumulation of viral RNA in cytoplasm. These are flagged as “atypical lymphocytes” by automated analyzer such as Sysmex XN 550. The newer analyzers can highlight such cells as high fluorescent lymphocyte count by assessing the increased cytoplasmic density with fluorescence.[19]

The present study also looked at utilizing the leukocyte flagging program of hematology analyzer as an inexpensive tool for screening dengue infection. In our study, higher proportion of patients with fever of more than seven days showed either atypical lymphocyte and/or blast flag (Table -3). In a study of hemocytometry on 93 adults with dengue, increased atypical lymphocytes and immature platelet fraction were characteristically noted by Sysmex XE-5000.[20] Clarice CSH et al evaluated the performance of Sysmex XS 500i in 530 patients with dengue and found higher atypical lymphocyte percentage (8.65 ± 12.09) in patients with severe disease than those without.[21] These atypical lymphocytes are identified by their higher nucleic acid percentage. Zhu et al proposed “lymph index” based on cell population data generated on automated hematology analyzer. This lymph index is calculated from lymphocyte volume, lymphocyte volume standard deviation and lymphocyte conductivity. While Lymph index in viral infections was higher with mean of 15.33, bacterial infections had lymph index of mean 11.32.[22]

5. Conclusion

To summarize our study showed that thrombocytopenia and raised hematocrit are not seen in all cases of dengue. Though, decreased absolute lymphocyte count is common in early days of fever, leucopenia is more likely seen in patients with fever of more than 3 days duration. Decrease in absolute neutrophil count is seen in dengue fever of four to six days duration. Leucocyte flags by analyzer such as Atypical lymphocyte", "Blast" and presence of reactive lymphocyte on peripheral smear evaluation can aid recognition of dengue infection. A hemogram with these abnormalities shall be referred to serology testing for timely diagnosis of dengue. The limitations of the present study include smaller size of study population and lack of comparison of hematological parameters in dengue with fever of other etiology.

6. Ethical clearance

Ethical clearance IEC/KRIMS/O/2019-20 obtained from Institutional Ethical Committee, KRIMS following full committee review.

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8. Conflict of interest

No conflict of interest to declare by the authors.

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