EVALUATION OF LH 750 VCS PARAMETERS AND LYMPH INDEX IN IDENTIFYING DENGUE FEVER

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ABSTRACT

Evaluation of LH 750 VCS parameters and lymph Index in identifying Dengue fever

Background: *Dengue is an acute viral infection which affects nearly 50-100 million people across many countries of the world. It is now threatening to become a major health problem across the globe. There is no specific treatment for Dengue and diagnosis depends mainly on serological testing and virus isolation from blood of patients. The major problem physicians face is to distinguish self-limiting Dengue fever from Dengue haemorrhagic fever.*

Aims and objectives: *The present study aims to evaluate the utility of VCS (volume, conductivity, scatter) parameters and lymph index in accurately predicting dengue fever. We retrospectively analysed data from 193 febrile patients coming to our diagnostic centre between August 2014 and October 2014 and calculated the lymph index in the patients positive for dengue infection by serological methods.*

Materials and Methods: *In this retrospective case control study, blood samples were collected from 193 febrile patients between August 2014 and October 2014. A simplified lymphocyte CPD(cell population data), the lymph index, was calculated as LV(lymphocyte volume) X LV- SD (standard deviation of lymphocyte volume) ÷ LC(lymphocyte conductivity).*

Results: *We observed a significant increase in the lymph index in dengue patients (≥13.6) as compared to controls (mean lymph index 10.32). The Sensitivity and Specificity in predicting dengue infection was 71.17% AND 78.05% respectively with a cut off of ≥13.6.*

Conclusion: *These results indicate that lymph index is a good hematological parameter for predicting dengue infection*

Key words: *Beckman Coulter LH 750, VCS parameters, Lymph index, CPD, Dengue fever.*

INTRODUCTION

Dengue is an acute febrile illness caused by an arbo virus that is endemic in more than 100 countries. It is transmitted mainly by Ades aegypti mosquito. There are four Dengue virus serotypes and infection with one serotype does not provide immunity against the other serotypes.[1] Now a days, dengue has emerged as a global health problem with 50 to 100 million infections occurring each year.[2]

Dengue fever presents with a wide variety of signs and symptoms. Normally, it is a self-limiting disease with fever, rash, headache, nausea, vomiting and bodyache as presenting symptoms. Sometimes, patients may develop severe symptoms due to increased vascular permeability and plasma leakage which may cause death.[3] Spontaneous bleeding may result in death of patients. There is no specific therapy, but timely initiation of supportive treatment can reduce the morbidity and mortality of severe cases to less than 1%.[4]

The early diagnosis of Dengue infection is crucial to adequately manage patients and monitor them for signs of spontaneous bleeding and plasma leakage so that clinicians can start early supportive treatment.[5,6,7] Initially dengue disease predominately affected the people living in tropical and subtropical zones. However, it is increasingly becoming a global health problem due to increased human migration. Weather, global warming and climate changes have resulted in zones where mosquitoes can breed and survive. To add to this problem, unauthorized urban development and poor waste management has increased the chances of water accumulation where mosquitoes can breed and survive.

Accurate diagnosis of dengue requires serological testing and identification of viral material in blood which is performed dominantly in the laboratories. Symptoms of dengue fever often overlap with other febrile illness like malaria specially in tropical countries. Thus the physicians are in a dilemma to distinguish between these febrile illnesses correctly and more importantly whether the dengue infection is self-limiting dengue fever or dengue haemorrhagic fever.

Dengue fever is a self-limiting disease where fever generally subsides within 3-7 days and patients recover fully. However, in Dengue haemorrhagic fever, petechiae, ecchymosis, epistaxis and other signs of spontaneous bleeding begin to appear after 3-5 days of fever. So an accurate and timely diagnosis of Dengue is imperative to initiate proper treatment .Unfortunately, the rapid screening tests have limitations depending upon their sensitivity and specificity. Virus isolation is more informative

but expensive and time consuming. Many studies have been conducted to discriminate Dengue from other febrile illnesses across the world. Sharma et al in 2014 assessed the diagnostic performance of LH750 hematology analyzer VCS parameters to identify malaria and dengue and distinguish them from other febrile illness[8]. Viral infections cause activation of lymphocytes, proliferation of undifferentiated lymphocytes and production of cytokines/ lymphokines. The VCS (volume, conductivity, scatter) technology of Beckman Coulter LH 750 haematology cell counter analyses over 8000 peripheral leucocytes in their "near native state". The cell volume is determined by direct current impedance, the conductivity of cytoplasmic chemical composition and nuclear volume is assessed by radiofrequency opacity. The cytoplasmic granularity and nuclear structure is assessed by laser beam to measure light scatter .[14]These are known as cell population data(CPD).

The present study aims to evaluate the utility of VCS parameters and lymph index in accurately predicting dengue fever. We retrospectively analysed data from 193 febrile patients coming to our diagnostic centre between August 2014 and October 2014 and calculated the lymph index in the patients positive for dengue infection by serological methods.

MATERIALS AND METHODS

Case selection: In this retrospective case control study, we collected blood samples from 193 febrile patients between August 2014 and October 2014 coming to Sampurna Sodani Diagnostic Clinic.All patients who had a history of fever from day 0 to day 7 were included in the study.

All patients had a positive Dengue Serology including either NS1 antigen, IgG or IgM (J.Mitra). Those patients who had high leucocyte counts were excluded presuming them to be bacterial infection.

There were 193 patients male(104) to female(89) ratio (1.16:1) with viral infections. There were 30 healthy controls. Majority of the patients were between 13 to 50 years of age. **Table I and Graph I** shows patient demographic data.

Data collection: Data was collected for each patient including CPD of lymphocytes which were generated by Coulter LH 750 (Beck Man coulter, CA). The LV (lymphocyte volume), LV-SD (SD of lymphocyte volume and LC (lymphocyte conductivity) were recorded. Other conventional parameters like total leucocyte count, differential count and platelet counts were noted down. A simplified lymphocyte CPD, the lymph index, was calculated as LV X LV- SD ÷ LC

All samples were analysed within 4 hours of specimen collection to avoid changes in cell morphology.

Pathogen Identification: All patients were tested for dengue infection by rapid serological tests (J. Mitra)

STATISTICAL ANALYSIS

All positive dengue patients either NS1, IgM or IgG were compared for CPD parameters with healthy controls and lymph index was calculated as

LV X LV- SD÷LC

The sensitivity and specificity of lymph index was calculated by XL STAT software.

RESULTS

1. **Changes in lymph index in dengue infection**: We retrospectively analysed the lymph index in 193 patients mean TLC (7.32+/-1.5) mean % neutrophils(58.94+/-10.4), mean % lymphocytes (30.49+/-4.3)and mean platelet count (185 +/-100). Out of 193 patients, 111 patients were positive for Dengue serology out of which 51 patients had normal platelet counts.

Table 2 shows mean SD of CPD data in dengue patients.

We observed a significant increase in the lymph index in dengue patients (≥13.6)as compared to controls (mean lymph index -10.32)

2. **Sensitivity and Specificity in predicting dengue infection:** The Sensitivity and Specificity in predicting dengue infection was 71.17% AND 78.05% respectively with a cut off of ≥13.6. These results indicate that lymph index is a good hematological parameter for predicting dengue infection.

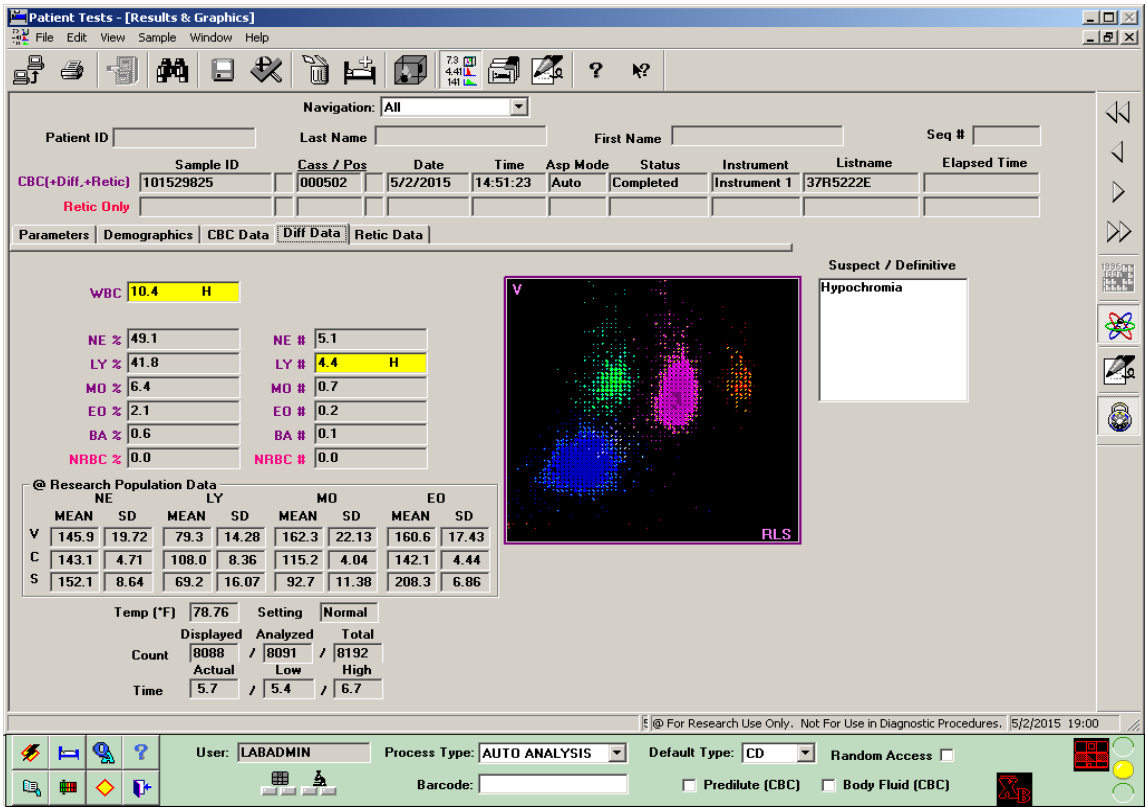


Fig. I: Normal CPD Data

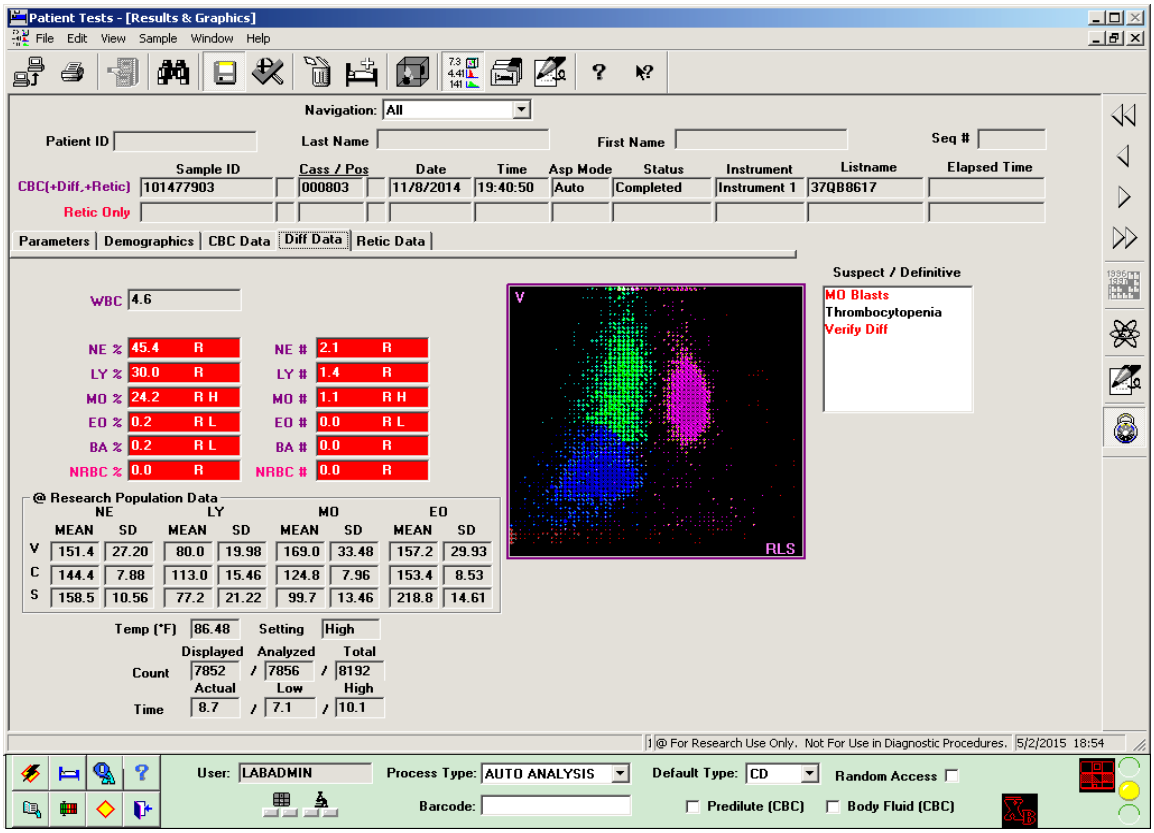


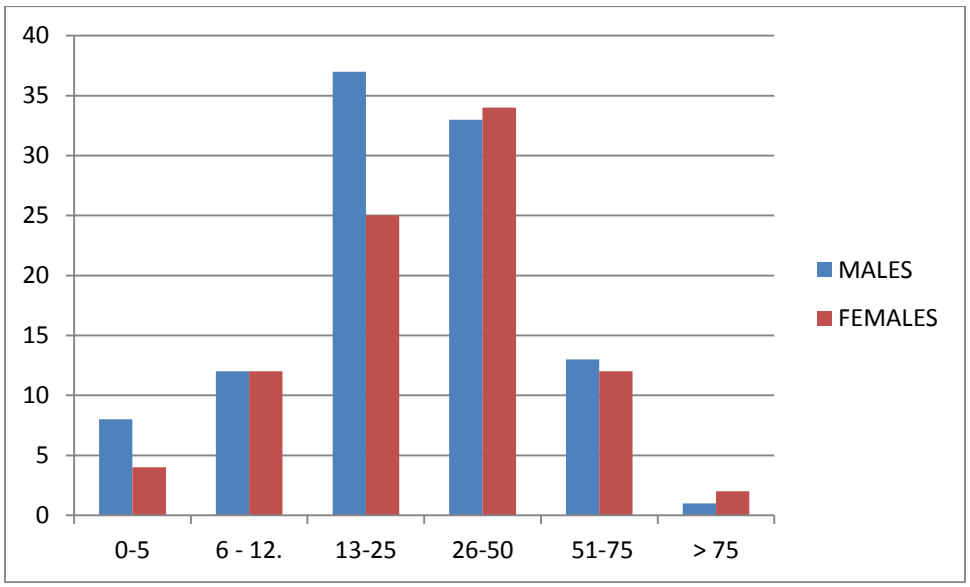
Fig. II: CPD Data of Dengue patient

Table I: Patients demographic data, total patients- 193

AGE in years	MALES	FEMALES
0-5	08	04
6-12	12	12
13-25	37	25
26-50	33	34
51-75	13	12
> 75	01	02
TOTAL	104	89

Table II: mean SD of CPD data of patients

	NE		LY		MO		EO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
V	152.27	25.13	85.02	17.59	178.92	24.46	153.80	19.17
C	144.54	7.01	114.40	13.03	123.04	5.49	146.16	6.62
S	151.57	11.99	78.12	19.06	97.82	10.67	204.29	8.01



Graph I: Showing demographics of patient population.

DISCUSSION

Beckman coulter LH 750 hematology analyzer provides quantitative data of leucocytes based on VCS (volume, conductivity, scatter) technology.[9] Attempts to diagnose dengue by haematology analyzers have been few in comparison to malaria, probably because unlike malaria infected red blood cells are unavailable to provide distinct patterns and also because the mononuclear cell changes are morphologically indistinguishable from those of other viral febrile illness like infectious mononucleosis.[10]

Two recent abstract from Puerto Rico have described a high area-under-curve for a dengue factor calculated by combining quantitative and morphologic monocyte information in an equation (% monocyte + SD of monocyte volume).[11,12] This factor was superior to the conventionally used platelet and leucocyte counts in differentiating dengue positive from dengue negative controls.

ZhuY et al in 2013 proposed a simplified lymphocyte CPD, the lymph index to diagnose viral infections and distinguish them from other bacterial infections. [13] The lymphocyte CPD included the mean volume (LV) with its standard deviation (LV-SD) and the conductivity (LC). The lymph index was calculated as

$$LV \times LV - SD \div LC$$

Using the lymph index cut off value of ≥ 12.92, they achieved 91.67%, Sensitivity and 97.2% specificity for diagnosing viral infections.

Rapid diagnosis of Dengue fever is vital for proper patient management. Although WBC and differentials including platelet counts may provide useful information the sensitivity and specificity of these parameters is usually poor. Other tests like ELISA and PCR techniques are time consuming, expensive and need a high level of technical support. The VCS technology used in LH750 analysers is able to generate the differential count based on cellular morphology using neither chemical reactions nor fluorescence.

Studies have shown alternations in lymphocyte CPD in viral infections.[15] LV and LV-SD, which represent lymphocyte size and size variation are significantly increased, LV is significantly decreased in viral infections as the nuclear/cytoplasmic ratio decreases due to increase in cytoplasmic chemical composition and nuclear volume.[16]

Our study had a relatively lower Sensitivity and Specificity as compared to the study conducted by ZhuY et al who showed a specificity of 97.2% and sensitivity of 97.1%. The higher sensitivity and specificity may probably be because they included all viral infections while our study aimed at only dengue infection. These results indicate that lymph index is a good hematological parameter for predicting dengue infection

CONCLUSION

In our study we demonstrated that lymph index is significantly increased in dengue patients. A lymph index cut off ≥13.6 we demonstrated a sensitivity of 71.7 % and specificity of 78.05% **.**Our study has some limitations. Firstly, the sample size is small because we analysed data from patients who had their complete blood count and Dengue serology done. There were many patients who had only their Dengue serology done but could not be included in the study because their CPD data was not available for analysis. Secondly, the small sample size may be because we included only those patients who had a positive Dengue serology.

Our findings may be useful for physicians as these parameters are easily obtained from LH 750 cell counter. There is no additional cost involved and CPD data are more accurate in evaluating morphological changes in leucocytes in viral infections than a peripheral blood examination which involves time and experience**.**

These results are promising in the ongoing quest to develop accurate reliable and rapid hematology analyzer-based diagnostic tests for this serious illness. A larger prospective study would be helpful in formulating decision rules and incorporating them in cell counters to provide flagging for Dengue infection so that laboratory work flow and quality of patient care improves.

Conflict of interest: none

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