Saudi Journal of Pathology and Microbiology (SJPM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

ISSN 2518-3362 (Print)
ISSN 2518-3370 (Online)

Utility of Cell Population Data as an Early Predictor of Dengue

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Original Research Article

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Article History
Received: 13.05.2018
Accepted: 27.05.2018
Published: 30.05.2018

DOI:
10.21276/sjpm.2018.3.5.1

Abstract: There is a need for a rapid and reliable test to predict dengue so that the patients are managed and monitored. Beckman Coulter LH series instruments provide data on leukocyte cell volume, V; conductivity, C; and light scatter, S which is useful in distinguishing viral and bacterial infections. This prospective study was undertaken in Chennai - India for a period of one and a half years. The cell population data for the dengue positive and negative patients were retrieved from the hematology analyzer Beckman Coulter LH780. Statistical analysis was performed using MedCalc for Windows version 15.0. Mann-Whitney test was used to compare the VCS indices between the different groups. Dengue positive, suspected and controls were 499, 493, 499 respectively. Male: female ratio is 1.4:1. The platelet count, standard deviation of volume and conductivity of monocytes, volume and percentage of lymphocytes and platelet counts were used to construct a tree model which distinguishes dengue from suspected cases with sensitivity, specificity and efficiency of 94.84%, 77.88% and 89.59% respectively. A classification tree was developed using changes in monocytes, lymphocytes and lymphocyte and platelet counts.

Keywords: Beckman coulter, Dengue, Lymphocytes, Platelet count, Thrombocytopenia.

INTRODUCTION

Dengue is a mosquito borne viral disease with symptoms ranging from mild febrile illness to dengue hemorrhagic fever with complications. The most common hematological abnormality is thrombocytopenia which may cause intracranial bleed. The conventional diagnostic tests are dengue IgM and NSA1 antigen, which requires a turnaround time of 24-48 hours, as the investigations are done in batches. Though clinical features and basic laboratory tests are done to prognosticate dengue, the sensitivity and specificity are very less [1]. There is a need for a rapid and reliable test to predict the disease so that the patients are adequately managed and monitored for signs of bleeding [2]. The early diagnosis of dengue infection is crucial to promote early supportive therapies, prevent the use of potentially harmful drugs and to assess the prognosis [3, 4].

Dengue has emerged as a global health problem with 50-100 million infections each year [5]. In 2012, a dengue epidemic occurred in India [6]. After this period, dengue continues to be endemic in Tamil Nadu with dengue positive patients being treated throughout the year, as out-patients and as in-patients.

There have been few reports on the application of cell population data, which is available as a research parameter in the hematology analyzers, in the diagnosis of dengue. Beckman Coulter LH series instruments provide quantitative data on leukocyte cell volume by voltage impedance, V; an estimate of cytoplasm nuclear ratio by radiofrequency conductivity, C; and cytoplasm granularity/ nuclear complexity by laser light scatter, S [7]. Utility of this cell population data in distinguishing viral and bacterial infections, have been studied and found to be useful. Also, a lymph index, which is a mathematical calculation of LV x LV – SD ÷ LC, has good sensitivity and specificity for diagnosing viral infection [7]. This lymph index was evaluated to discriminate dengue from other viral infections and found to have low sensitivity and specificity [8]. A dengue factor calculated by combining quantitative and volume of monocytes in an equation has been found to be useful in discriminating dengue positive from dengue negative patients [9]. A study done in Chandigarh also showed promising diagnostic utility of cell population data in identifying dengue from other illness**.** They developed a cell population data based malaria-vs.-control factor along with a dengue-vs.-control factor and febrile control-vs.-malaria/dengue factor to

distinguish viral fevers as a close mimic of malaria on Beckman Coulter LH750 instrument [10]. Multicentric researches needs to be done before validating the utility of cell population data as a reportable or predictive parameter for dengue fever.

With this relevant literature, we aimed at analyzing the hematological and VCS indices with a view to generate algorithms and decision trees which could be used to flag in the laboratory information system (LIS) to discriminate dengue from other diseases or predict dengue thereby alerting for further testing and vigilant watch.

MATERIALS AND METHODS

Study setting

This case control study was done among the patients admitted to our tertiary care hospital in Chennai with the diagnosis of dengue. The duration of the study was for one and a half years, from June 2015 to December 2016.

Study population

All the patients who were admitted to our hospital with a diagnosis of dengue during the study period were selected for the study. A total of 499 dengue cases, 493 suspected cases and 499 controls participated in this study.

Ethical approval and informed consent

Approval from the Institutional Ethics Committee was obtained prior to the commencement of the study. Each participant was explained in detail about the study and informed consent was obtained prior to the data collection.

DATA COLLECTION

Samples for complete hemogram and dengue serology were sent at the same time when dengue was suspected. Laboratory records for dengue serology requests the results were retrieved. Dengue IgM and/or NSA_1 antigen positive samples were taken as dengue positive. Samples negative for dengue IgM and/or NSA_1 antigen were taken as dengue negative febrile illness patients. The Cell population data, platelet count and differential count for the positive and negative patients were extracted from the hematology analyzer Beckman Coulter LH780. The platelet count, differential count, and cell population data of master health checkup samples were retrieved from the hematology analyzer and were taken as controls.

Operational definitions

- To assess the diagnostic performances of various parameters Receiver operating characteristics (ROC) curve analysis with calculation of the area - under - the - curves (AUC was used). 95% confidence interval was maintained.
- Youden index was calculated to assess the sensitivity and specificity of each parameters in distinguishing dengue. Analysis was done between dengue positive (Pos.) vs. dengue suspected (Neg.), dengue suspected (Neg.) vs. normal (Nor.) and dengue positive (Pos.) vs. normal (Nor.) for each parameters.
- Platelet, SD_V_LY, SD_V_MO, MN_C_MO, Lymph % were found to be the most informative ones and were used to perform statistical calculations and construct a tree model to predict dengue.

Data analysis

The data weas tabulated in Microsoft excel data spread sheet 2010. Statistical analysis was performed using MedCalc for Windows version 15.0 (MedCalc Software, Ostend, Belgium). Mann – Whitney test was used to compare VCS indices and other parameters between different groups.

RESULTS

This study was done among 499 dengue positive patients, 493 suspected patients and 499 normal controls. The mean age of dengue positive patients was 26.8 years, and that of negative was 36 years. The mean age of controls was 47.7 years. The mean male: female ratio for patients and controls was 1.4:1.

Individual VCS parameters, platelet count, lymphocytes and monocytes were compared between dengue positive and negative groups and dengue positive and control groups. Low sensitivity and high specificity was noted when parameters are taken individually to predict dengue. Hence the five parameters namely PLT, SD_V_MO, SD_V_LY, MN_C_MO, Lymph% were found to be the most informative ones. Based on the data analysis of these parameters, a tree model had been constructed to predict dengue. The tree model is shown in figure 1. The tree has 2 splits and 13 terminal nodes. The initial split was based on the conductivity of the monocytes with a cut off of 105.75. Platelet counts of 1.23 lakhs/cumm were utilized for one arm and SD_V_MO was utilized in the other arm. Repeated use of the five parameters helped to clearly demarcate non dengue and dengue patients with a population of grey zone patients who can be either dengue positive or negative.

For dengue vs. suspected, the tree could predict dengue with a sensitivity of 94.84%, specificity of 77.88% and efficiency of 89.59%. The false negative and positive cases were very few. The grey zone patients were 63%. For dengue vs. control, 50.5% of the patients were in the grey zone and true positive cases were predicted with a sensitivity of 94.845, specificity of 97.5% and efficiency of 96.14%. In total the corrected diagnosis for 47.6% (239+234÷994=47.6). The analysis was done on the sample population based

on the tree model and the performance of the tree model is shown in Table-1.

Table-1: Performance characteristics of the tree model

	Dengue vs suspected				**Dengue vs control**			
Grey zone, among them:	621				502			
Suspected / control	377				258			
Dengue	244				244			
Classified patients:		**sensitivity**	**specificity**	**efficiency**		**sensitivity**	**specificity**	**efficiency**
True Positive	239	94.84	77.88	89.59	239	94.84	97.50	96.14
False negative	13				13			
True negative	88				234			
False positive	25				6			
TOTAL classified	365				492			
Total	986				994			
Total efficiency=% of patients correctly classified from all patients	33.1643002				47.58551308			
Size of grey zone, % of all patients:	62.98174442				50.50301811			

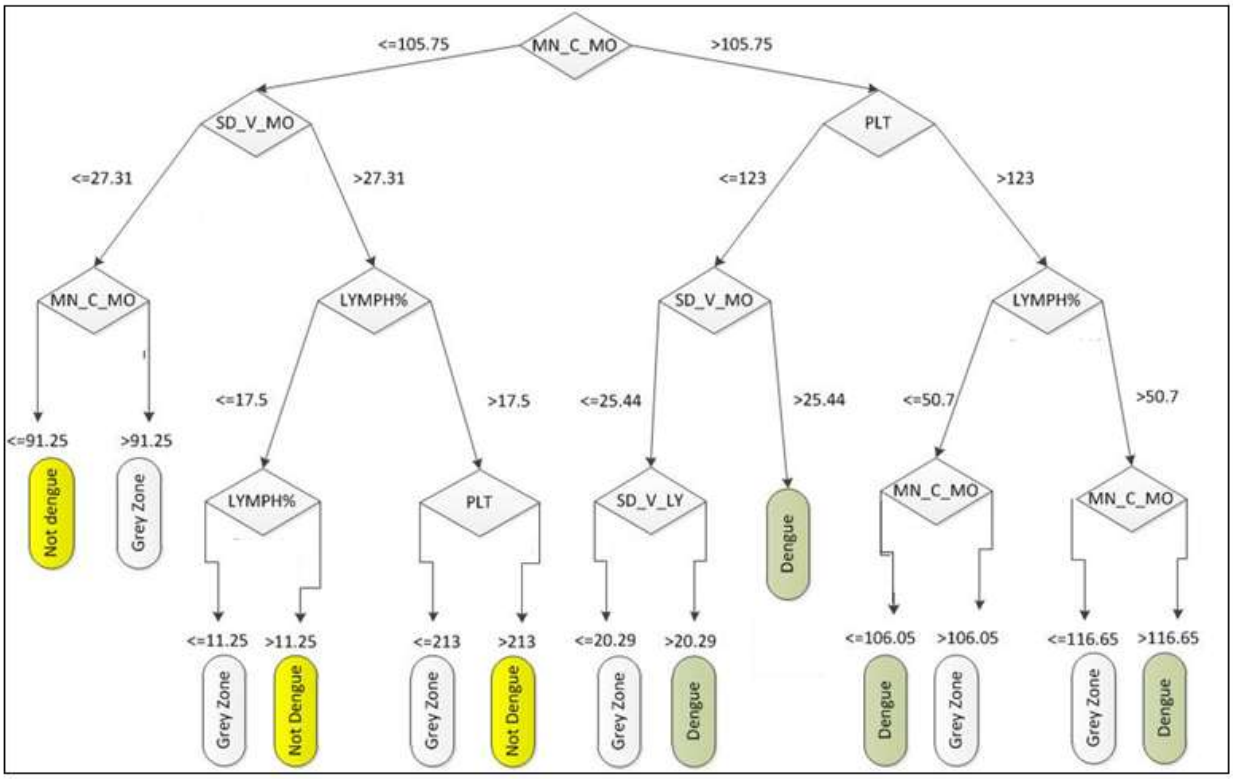

Fig-1: Tree model to predict dengue with platelet count and CPD

DISCUSSION

Predicting cases of dengue infection and differentiating it from other viral and bacterial illnesses help in vigilant watch and supportive therapy. Hence there is a need for a predictive parameter with a short turnaround time. Beckman Coulter LH780 Hematology analyzer provides quantitative and qualitative data of leukocytes. It is well known that in viral infection the lymphocytes, monocytes and platelets were more involved than the granulocytes. Viral infection causes proliferation and activation of lymphocytes with antibodies and cytokines being secreted [11]. The cytoplasmic alterations and morphologic changes occur in the activated lymphocytes. These changes could be detected by VCS. Significant changes have been noticed in hepatitis B virus infection and other viral infections [7, 12]. The SD_V_LY and Lymph% were used in our study to construct the tree model. Lymphocyte conductivity was not used, as there was no significant difference found in dengue positive patients.

In an early study, a malaria factor, utilizing standard deviations of monocytes, volume and size of lymphocytes was achieved for detecting malaria parasite [13, 14]. Sharma P *et al.*, have incorporated mean corpuscular hemoglobin concentration and neutrophil percentage along with mean volumes of lymphocytes and monocytes and SD of monocyte volume to differentiate dengue and malaria from other febrile illness [10]. These authors have also included platelet count and percentage of lymphocytes and standard deviation of lymphocyte conductivity to distinguish dengue and constructed a tree model. In our study also, the standard deviation of monocyte volume, platelet count and the mean conductivity of monocytes showed significant changes and were included to construct the tree model.

A dengue factor was developed combining quantitative and morphologic details of monocytes as an equation to diagnose dengue by Lopez R and Soto RM *et al*., [10, 11] A study from Kerala in India, had shown hypogranulation of neutrophils and increased monocyte count, mean volume and SD of monocytes in dengue patients [13].

In this study, utilizing the VCS parameters available in Beckman Coulter LH780, the values of cell population data which showed changes and platelet count, have been utilized to arrive at a classification trees with two splits and 13 terminal nodes. The performance of the classification tree has been found to be 89.59% efficient in discriminating dengue vs. suspected, and is 96.14% in discriminating dengue vs. normal controls. This tree if formulated in the software, the dengue cases may be predicted within a turnaround time of 1 hour so that the clinicians can be alerted to have a vigilant watch over these patients. However the number of patients in the grey zone was high. These patients can also be advised to be alert for warning signs. The quantitative and morphological parameters utilized in the tree model are readily obtained by hematology analyzer during automated leukocyte differentials without involving any additional cost. When compared with microscopic examination of peripheral smear, these parameters are more objective and accurate. These if incorporated in the instrument flags would allow improvements in laboratory work flow and qualitative patient care [15].

CONCLUSION

A classification tree was developed using standard deviation of volume and conductivity of monocytes, standard deviation of lymphocytes, percentage population of lymphocytes and platelet counts. We have observed that this tree has good efficiency to predict dengue. Implementation of this tree in prediction of dengue in clinical practice is of key in prevention and early case management of dengue. Utilization of this tree model involves no additional cost and is significantly accurate.

DECLARATION

Funding

This study was a part of Summer Research Fellowship of Sri Ramachadra University.

ACKNOWLEDGEMENT

I profusely thank the Chancellor and the management of Sri Ramachandra University for awarding the Summer Research Fellowship to carry out this study. Dr. Elena Sukhacheva, PhD, provided scientific and methodology – related inputs at various stages of this study. Mrs. Sandhya Bastian, Product Manager - Hematology, Beckman Coulter Inc. for coordinating the statistical work up. The technical staffs of the Hematology and Clinical Pathology division, Sri Ramachandra Laboratory Services are acknowledged for their help in sample collection and processing.

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