ORIGINAL ARTICLE

Clinical Utility of Extended Monocytes Parameters as a Screening Tool in Suspected Dengue Infection

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ABSTRACT

Introduction: Increased monocyte percentage and monocyte anisocytosis were suggested as new markers for dengue fever detection. This study aims to investigate and evaluate monocyte volume standard deviation (MoV-SD) and monocyte percentage (Mono %) parameters using Coulter automated haematology analyser as screening parameters in discriminating between dengue infection and other febrile illness. **Methods:** A cross-sectional laboratory analysis using suspected dengue fever patients were included in this study. The study was conducted in the Department of Pathology, Hospital Tuanku Jaafar Seremban from June 2016 until June 2017. Patients were classified into dengue positive and dengue negative based on dengue IgM and NS1 result. The diagnostic performance of MoV-SD and Mono % was analysed by receiver operating characteristic (ROC) curve analysis. The cut-off value of the MoV-SD and Mono % was determined and evaluated with the validation group. Chi-square test was used to assess the association between the parameters. **Results:** 88 (48.4%) from 182 samples were confirmed to have dengue infection. ROC curve analysis showed Mono % at cut off value of 10.5 % with area under the curve (AUC) of 0.869 with 84.1% sensitivity and 84% specificity (95% CI: 0.812-0.925) and MoV-SD cut off value at 22.2 (AUC 0.776, 80.7% sensitivity, 61.7% specificity, 95% CI: 0.709-0.843) are an excellent parameters in separating dengue positive and dengue-negative patients. A cut-off value of 10.5 of Mono % and 22.2 of MoV-SD were applied to the validation group showed 83.1%, 66.4% sensitivity and 84.9%, 77.3% specificity respectively. **Conclusion:** MoV-SD and Mono % parameters are a potential parameter for the screening of dengue infection in acute febrile illness patients with good specificity and sensitivity.

Keywords: Monocyte percentage, Monocyte volume standard deviation, Dengue infection

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INTRODUCTION

Dengue is highly prevalent worldwide and the incidence keeps increasing yearly. Malaysia reported the highest cases in between 2014 and 2017 with 237 death in 2016 (1). It is caused by viruses named DENV1, DENV2, DENV3, and DENV4. There is no specific pattern can be identified throughout the year in relation to the serotype. All serotype can cause severe infection and fatality.

Clinically suspected dengue fever are challenging to be differentiated from other febrile illnesses since many of the signs and symptoms are overlapping. Dengue can be diagnosed using several investigations by detection of the virus antigens or antibodies or a combination of these techniques. However, due to many factors such as variable in sensitivities and specificities of the tests (2), lacking proper collection and storage techniques, scarcity of technical expertise, non-availability of the infrastructure and delay in obtaining the results leads to limitations for proper diagnosis (3).

There are increasing publications demonstrated the benefits of clinical applications in leukocytes morphological parameters changes or cell population data (CPD) in various disorders. CPD was found to be comparable to microscopic leukocyte morphological evaluation. Numerous infections including dengue can caused changes in leukocyte morphology. Automated cellular indices based on the changes in leukocytes during infection derived from haematology analysers may offer a rapid distinction between dengue and other febrile illness (4). Automated haematology analyser from Coulter utilised the CPD through volume, conductivity and scatter (VCS) technology. It determines the intrinsic biophysical properties of peripheral leukocytes. It uses direct current impedance to measure cell volume (V) for cell size, radio frequency opacity conductivity (C) for the internal composition of each cell and light scatter

(S) for cytoplasmic granularity and nuclear structure (5). This technology is capable of automatically quantifying leukocytes morphology characteristics in which each mean and standard deviation (SD) of the parameters can be obtained. In the past few years, many studies have utilised these CPD data for the benefit of the patients without additional cost.

The diagnostic accuracy to differentiate between various febrile illness and dengue fever in a clinical setting will certainly improve with the use of the haematology analysers with VCS technology since it evaluates both the numerical parameter as well as the morphology features of leukocytes (6).

Monocytes have been implicated in the pathogenesis of dengue whereby it acts as the primary target of dengue viruses (7). Monocyte CPD showed specific changes in viral fever particularly in dengue fever thus making it as a potential parameter to be used in discriminating dengue fever and other febrile illness. Increase in monocytes number and variation of size and shape of the monocytes during dengue infection can be detected using monocytes percentage (Mono %) and monocytes volume standard deviation (MoV-SD). Monocytosis and monocyte anisocytosis even present in dengue-infected patients with unremarkable haematological profiles, which make the correct diagnosis of dengue infection could easily be overlooked (8). These parameters were found to have good sensitivity and specificity in the detection of dengue infection (8). Therefore the application of these parameters into clinical usage will certainly offer many advantages (6). The aim of this study was to investigate and evaluate the usefulness of these monocytes parameters to be used as a screening parameter in the detection of dengue infection.

MATERIALS AND METHODS

Study population

This cross-sectional study was conducted at Hospital Tuanku Ja'afar, Seremban, Negeri Sembilan from June 2016 to June 2017. Samples received in the laboratory from adult patients with acute febrile illness (less or equal to seven days) suspected to have dengue fever and had their samples sent for full blood count on the day of admission, dengue IgM and Non-Structural Protein-1 (NS1) were recruited into the study by simple random selection. Patient's data were accessed from the patient information system and recorded. Patients with incomplete data were excluded. Patients were divided into two groups, dengue positive and dengue negative based on dengue IgM and dengue NS1 antigen result. Dengue positive patients were defined as dengue IgM and dengue NS1 antigen positive, whereas dengue-negative patients were defined as dengue IgM and dengue NS1 antigen negative result.

For the validation group, another set of patients, from the same hospital who presented with acute febrile illness (less or equal to seven days) with unknown dengue status were chosen from January to June 2017.

Analytical methods

Full blood count parameters including extended monocyte parameter were performed by DxH800 (Beckman Coulter, USA) automated haematology analyser. Cell population data on monocyte parameters [monocyte percentage (Mono %), monocyte volume standard deviation (MoV-SD)] were obtained from the system.

Data on dengue serology IgM and dengue NS1 antigen were retrieved from the record. Dengue serology IgM-capture ELISA (Euroimmune, Germany) antibody detection was performed using serum from suspected dengue cases combine with anti-human IgM antibodies that attached to the polystyrene surface of the microwell test strips. Dengue NS1 antigen (Euroimmune, Germany) detection was using patient's serum incubated into the microplate wells sensitised with anti-NS1 monoclonal antibodies.

Statistical analysis

Statistical analyses of the data were performed using the IBM statistical package for the social sciences (SPSS) version 25. The diagnostic performance of Mono % and MoV-SD in discriminating between dengue positive and dengue negative were assessed with receiver operating characteristic (ROC) curve analysis. ROC and area under the ROC curve (AUC) were used to determine the optimal cut-off value for Mono % and MoV-SD. A p-value < 0.05 was considered statistically significant. The diagnostic performance of Mono % and MoV-SD was evaluated with the validation group. The best fit value obtained by the ROC curve was tested in this validation group and justified by dengue IgM and NS1 antigen. True positive, true negative, false positive and false negative rates were determined. Sensitivity and specificity in the validation group were obtained.

Pearson chi-square test was done to determine the association between Mono % and MoV-SD with dengue IgM and dengue NS1 antigen and risk estimation was determined.

Ethics

The study was approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-16-2607-33696) and the Ethics Committee for research involving Human Subjects, University Putra Malaysia [FPSK(FR16)P022].

RESULTS

A total of 182 samples of suspected dengue cases, 88 (48.4%) samples were dengue positive whereas 94 (51.6%) were dengue negative. Median (IQR) age for

all cases was 29 (31) whilst for the dengue positive, the median (IQR) age was 30 (26). Within the positive dengue cases, it showed male-predominant 48 (54.5%) compared to female 40 (45.5%). Majority of the dengue positive samples were from Malay 56 (63.6%) followed by Indian 15 (17.0%), Chinese 8 (9.0%) and others 9 (10.2%) (Table I).

Table I: Characteristics of the study population and demographic distribution among dengue positive patients

Variable	n	%
Age median (IQR)=29 (31)		
Dengue infection		
Positive	88	48.4
Negative	94	51.6
Age median (IQR) = 30 (26)		
Gender		
Male	48	54.5
Female	40	45.5
Race		
Malay	56	63.6
Chinese	8	9.0
Indian	15	17.0
Others	9	10.2

Based on the classification of dengue positive and dengue negative cases, the diagnostic performance of monocyte parameters was generated using ROC curve analysis (Figure 1). The optimal cut off value of Mono % of more than 10.5 resulted in the best AUC 0.869, 84.1% sensitivity and 84% specificity with 95% CI (0.812-0.925) (Table II). Whereas the cut-off value of MoV-SD more than 22.2 resulted in an area under the curve (AUC) 0.776, 80.7% sensitivity and 61.7% specificity with 95% CI (0.709-0.843) (Table II).

In the validation group (another n=182), when a 10.5 cut-off value for Mono % was applied, 84.1 % were correctly classified into the dengue positive and dengue

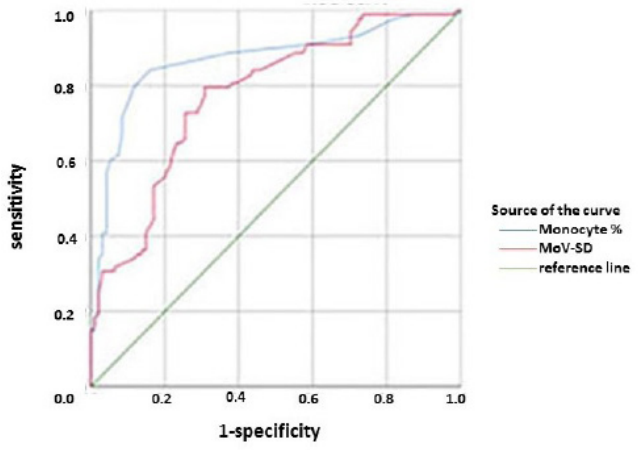

Figure 1: ROC curve for Mono% and MoV-SD for detection of dengue infection among suspected dengue patients

Table II: Optimal cut-off value of monocytes parameters based on ROC curve analysis

	Cut off value	AUC*	Sensitivity	Specificity	CI#	*p*-value
Mono %	> 10.5	0.869	84.1%	84.0%	0.812-0.925	<0.001
MoV-SD	> 22.2	0.776	80.7%	61.7%	0.709-0.843	<0.001

* Area under curve
\# Confidence interval

negative with 83.1% sensitivity and 84.9% specificity. Whereas when 22.2 cut-off value for MoV-SD was applied, 70.1 % were correctly classified into the dengue positive and dengue negative with 66.4 % sensitivity and 77.3 % specificity. Chi-square test showed there was a significant association (p<0.001) between the Mono % and MoV-SD with dengue serology. The findings are summarised in Table III.

Analysis by risk estimation also showed a relative risk of 5.5 times (95% CI 3.378 – 9.302) where it is more likely for those with Mono % more than 10.5 % to have dengue positive compared to those with Mono % less than 10.5 % whilst for those with MoV-SD more than 22.2, the relative risk increased by 2.9 times (95% CI 1.887- 4.542) to have dengue positive compared to those with MoV-SD less than 22.2.

Table III: Sensitivity and specificity of Mono % and MoV-SD in control group and their association with dengue serology

	Dengue		X^2 statistic (df)	*p*-value
	Positive, n (%)	Negative, n (%)		
Mono % ≥ 10.5	74 (83.1)	15 (16.9)	84.4 (1)	<0.001
Mono % < 10.5	14 (15.1)	79 (84.9)		
MoV-SD ≥ 22.2	71 (66.4)	17 (22.7)	33.7(1)	<0.001
MoV-SD < 22.2	36 (33.6)	58 (77.3)		

DISCUSSION

Early detection of dengue infection and differentiation from other febrile illnesses is important. The peripheral blood count is the first and basic investigation requested in any febrile illness. The usual findings of leukopenia and thrombocytopenia are expected in dengue fever but lacking those findings might mislead the diagnosis and causing a delay in confirming the diagnosis.

In dengue infection, changes in the number of leucocytes are accompanied by morphological changes as well. These changes are reflected in the VCS parameters and have been found to be useful for early prediction of bacterial infection, viral infection, malaria and dengue virus infection (9, 10, 11).

Monocytes are important in the pathogenesis of dengue infection as it is the most active sites of virus replication during infection. As a result, there will be a profound variation of size in monocytes which might due to strong immune stimulation and it reflected by MoV-SD (8). A study done by Babu Raj et al. has demonstrated that monocyte volume and count is increased in dengue

infection even with normal white blood cells and platelet count (12). Our study also showed there was a significant increase of monocyte number and volume in dengue infection in which 83.1% of those with Mono % more than 10.5% had dengue positive and 66.4% of those with MoV-SD more than 22.2 had dengue positive. Many previous studies have demonstrated monocytosis associated with dengue infection (8, 13, 14).

These monocytes parameters are generated during automated differential full blood count analysis typically under two to three minutes without additional specimen requirements or cost involved and fast result compared to dengue IgM serology and NS1 antigen. Dengue IgM can only be sent after day 4 onset of symptoms otherwise the chances of false negative is high. Based on our hospital policy, the turnaround time for dengue IgM serology and NS1 antigens are three days whilst the actual test to be completed took two hours. Therefore these monocytes parameters are at an advantage to be used as a screening parameter in suspected dengue infection. Hence high index of suspicion of dengue infection with the used of monocytes parameters is readily available right before the confirmatory test of dengue result are ready. Since dengue is endemic in developing countries including Malaysia, these monocytes parameters offer a great advantage (5). In fact, these parameters not only can be used as a reliable screening for dengue infection but also for follow up as the value should be lesser than the cut-off limit.

Based on the ROC curve analysis, we have demonstrated at a cut-off point Mono % of 10.5% resulted in good sensitivity (84.1%) and specificity (84.0%) with AUC of 0.869 (95% CI: 0.812-0.925, p<0.001) whereas MoV-SD at cut-off point of 22.2 have resulted in 80.7% sensitivity and 61.7% specificity (AUC: 0.776, 95% CI: 0.709-0.843) in discriminating between dengue positive with the other febrile illness. Our AUC for both parameters indicate that it is a good parameter in discriminating between dengue fever and other febrile illness. We found a better AUC for both parameters compared to Rabsarry et al, in which they found AUC of 0.71 and 0.74 for Mono % and MoV-SD respectively (5). Whereas, another study in India, the authors only found AUC for Mono % of 0.61 with a sensitivity of 63.6 % and specificity 61.2 %. For MoV-SD they found AUC of 0.58 with sensitivity 66.0 % and specificity of 55.6% (15). Our sensitivity and specificity were much improved compared to those findings. However, Aniwatangkoora et al. found the best result for MoV-SD in which at the cut-off 23.8 had resulted in AUC of 0.996 with 95% sensitivity and 100% specificity when comparing between control and dengue positive (16). The variation could be due to the different population in the studies as some analytes determined in the clinical laboratory may vary during an individual's lifetime because of biological inherency (22).

At the cut-off of 10.5 for Mono % and 22.2 for MoV-SD, these parameters seem to have good sensitivity and specificity to be used as a screening parameter for dengue fever. Therefore, any febrile illness presented higher than these value should have a high suspicion of having dengue fever. However, our cut-off value varies from other reported diagnostic cut-off value for dengue fever. Sohaib et al. found cut-off > 8.85 for Mono % and >24.79 for MoV-SD (15). This value is almost close to the cut-off in the current study. Whilst other study demonstrated a cut-off of >15.7 for Mono % and 27.6 for MoV-SD (5). This variation shows the need to establish cut-off limits for different populations. It has been shown before that biological variation was presence within the CPD parameters measured with VCS technology (21). The documentation of variation is important as an essential prerequisite in the development of any new application clinically (22).

It has also been shown that there was a statistically significant association between the monocytes parameters with dengue infection. Approximately, 74 (83.1%) of those with Mono % more than 10.5% had dengue positive and 71 (66.4%) of those with MoV-SD more than 22.2 had dengue positive. Whereas Libre Berness et al. found MoV-SD more than 30 has an association with dengue infection and monocyte percentage was only as a confounder factor (17). Both Rabssary et al. and FR Suman et al. also found a significant association between these monocyte parameters and dengue infection (8, 18). Hence these parameters are useful for screening of dengue infection. We also found that patient with Mono % more than 10.5 % have five times a higher risk to have dengue fever and MoV-SD of more than 22.2 have almost three times increased the risk to have contacted with dengue infection. It has been shown before, depletion of monocytes in a murine model of dengue infection resulted in a tenfold increase in systemic viral dengue titers thus showing the importance of monocytes in dengue infection (7) whereas many other studies (19, 20) have demonstrated the association of monocytosis with dengue infection however there were no risk estimation were defined especially with new parameter MoV-SD like in this study.

In the validation group, when cut-off value Mono % >10.5 was applied, 74 cases were correctly classified into dengue positive and 79 cases dengue negative. It resulted in the sensitivity (83.1%) and specificity (84.9%) almost similar to ROC curve analysis. Whereas for MoV-SD, the cut-off value of 22.2 was applied had resulted in 71 cases into dengue positive and 58 cases dengue negative. The sensitivity (66.4%) had reduced whilst the specificity (77.3%) had improved compared to the value achieved by ROC curve analysis. With the improvement in the specificity achieved in the validation group, it showed the validity of this parameter as a screening

parameter. We believed our study has broadened the horizon in the literature of dengue illness.

However since it was a cross sectional study using previous hospital data available, the variation in the clinical and laboratory features that took place during the course of illness could not be assessed which served as a limitation in this study. Secondly, the data is from only one region therefor generalisation of results is difficult and samples from other parts of the state need to be studied prospectively.

CONCLUSIONS

Our current study observed a significant association between MoV-SD and Mono % parameters in defining positive dengue infection. These parameters have the potential to be used as a screening test to delineate dengue fever from other febrile illness and has promising clinical utility to be utilised.

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