Original article:

Comparison of dengue and other febrile illnesses in patients admitted to Teaching Hospital-Peradeniya

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Abstract:

Background: Dengue is a severe and notifiable infection in Sri Lanka and other tropical countries. There are other febrile illnesses,which have the same symptoms as dengue. Identifying early changing markers in blood and their changing patterns in dengue and other febrile illnesses will help to differentiate dengue patients from other febrile illnesses.It is important for the early recovery and management of the dengue patients. *Methods:* Dengue suspected 288 patients were selected from adult medical wards in Teaching hospital-Peradeniya, after initial screening process. Out of whole population, 56% were RT-PCR positive for dengue and RT-PCR negative population (44%) was named as other febrile illnesses. Results of variations in liver enzymes, full blood count (FBC), demographic and hospitalization details in both groups were obtained from hospital records. *Results:* Male predominance and highly affected young population were detected in both groups. Number of days from onset of fever to admission to the hospital is lower and hospitalization period is higher in dengue than other febrile illnesses. On admission, higher level of aspartate transaminase, significant lymphopenia and higher mean cell volume (MCV) were noticed in dengue patients. On discharge, lower total white blood cell count, lower absolute lymphocyte count, lower platelet count and increased levels of transaminases were detected in dengue compared with other febrile illnesses.*Conclusion:* From dengue suspected admissions, 44% was due to other febrile illnesses which was very difficult to distinguish by symptoms of dengue. Young patients with male predominance is common in dengue as well as in other febrile illnesses. Recovery of cytopenia in FBC and normalization of liver transaminases were lower in dengue.

Keywords : Dengue; other febrile illnesses; RT-PCR; liver function tests; full blood count

Introduction

Dengue is an epidemic disease present in more than 100 countries in the world. Especially as tropical countries, Sri Lanka, India and Pakistan have better environmental conditions which are more preferable for dengue vector[1]. Annual Health Bulletin has shown that in 2012, 44 456 infected dengue patients were reported in Sri Lanka[2]. During first 42 weeks of 2016, 43 895 cases of dengue fever were reported[3]. When considering the whole population in Sri Lanka,

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dengue affected amount is highly remarkable.

Dengue is categorized as asymptomatic infection, dengue fever (DF), DHF and dengue shock syndrome (DSS) according to the WHO criteria[1]. Dengue infection is caused by four serotypes as DEN 1 – 4 [4].

Real time Polymerase chain reaction (RT PCR), enzyme-linked immunosorbent assay (ELISA), IgM, IgG, Nonstructural protein (NS1) antigen rapid strip test are used to diagnosis of dengue. As supportive parameters, platelet count, PT (Prothrombin Time) and APTT (Activated Partial Thromboplastin Time) are used. Hematocrit and total WBC count are mostly used for the monitoring of dengue[5]. It is shown that false positive results for dengue found in some diagnostic methods[6]. Detection of viral nucleic acid is vital to prevent false positive results[7].

Similar dengue symptoms as fever, headache, joint pain, muscle pain and rash are observed in other febrile illnesses as Chikungunya/Zika viral infections[8]. These infections are also transmitted by the dengue vector, *Aedesaegypti* and *Aedes albopictus*[9].

This study was based on changes in demography, hospitalization details and blood parameters (Liver transaminases, FBC) among patients with RT-PCR positive dengue and RT-PCR negative OFI. Dengue and OFI express similar clinical symptoms. In Sri Lanka, more patients are admitted into hospitals in dengue epidemics by suspecting dengue on symptoms. Because of the no availability of reliable investigations like RT-PCR in more hospitals, all patients are considered as dengue. Because of that, more hospitals are packed with patients indengue epidemics and the real dengue patient is not getting special attention and treatments.

There are facilities in most hospitals including rural to perform FBC and liver function tests. By following recent research studies, liver transaminases and some FBC parameters are believed as more preferable, benefited investigations as markers for the management of dengue patients. Therefore, early finding of significant differences in blood parameters between these two categories were more important for the patient management and monitoring. It will be also helpful for the identifying vital effects of dengue and early recovery. This is more useful for clinicians and other health professionals to prevent wastage of sources for unnecessary investigations.

Methodology Study population

Ethical clearance

Ethical clearance for the study was obtained from the Ethical Review Committee of Kotelawala Defence University. Hospital permission was obtained from the Director, Teaching Hospital- Peradeniya. Dengue suspected 288 patients were selected from adult medical wards of Teaching Hospital- Peradeniya from 01st of May 2016 to 31st of August 2016 and categorized as RT-PCR positive dengue (162 patients) and RT-PCR negative other febrile illnesses(126 patients).

Inclusion and exclusion criteria

Adult patients (14years<) with any two dengue suspected symptoms as headache, high fever (more than 3 days), fatigue, joint pain and muscle pain, abdominal pain with a low platelet count ($\leq 100,000$ / mm3) were included in dengue suspected population. Screening process was done by the medical officer in outpatient department (OPD).

Planned subgroup analysis

For each suspected dengue patient, following tests were performed routinely (daily in hospitalization period). All information about age, gender (demographic data), days from fever onset to admission and hospitalization period of whole study population were obtained from hospital records. Study didn't affect to any treatment of patients.

Real time RT-PCR test was performed in Analyzer- Applied Biosystems Quant Studio 6flex Real Time PCR. ALT and AST were done in fully automated analyzer - Beckman Coulter AU 480 by using kinetic method. Full blood count (FBC) was done by 7-part analyzer - Mindray 6800.

Methods of statistical analysis

Collected data was analyzed statistically by using SPSS software by dividing the study population into two groups as patients with RT-PCR positive dengue and patients with RT-PCR negative other febrile illnesses. The mean values of investigated parameters were used to compare changes in both groups. Distribution of age, gender, mean number of days from fever onset to admission and hospitalization period of both groups were obtained by using descriptive statistics.

By using descriptive statistics and p value by independent samples t test, mean values of ALT, AST and FBC parameters of patients were compared between both groups. For each group, all parameters were analyzed on admission and on discharge to detect

whether they are normalized within hospitalization period.

Results

Out of the 288 suspected dengue population, 162 (56%) were real time RT-PCR (Reverse transcriptase –polymerase chain reaction) positive dengue patients and 126 (44%) were real time RT-PCR negative patients named as other febrile illnesses. Most dengue patients in population were belonged to DEN-1(59.6%) serotype (*Table 01*).

Table 01: Distribution of patients with dengue and other febrile illnesses

Category	Count	Percentage	Serotype	Percentage
Dengue	162	56%	DEN-1	59.60%
			DEN-2	25.50%
			DEN-4	14.90%
Other febrile illnesses	126	44%		

P < 0.05 *, p < 0.01 **, p < 0.001 ***

Table 02: Comparison of descriptive statistics of demographic parameters and hospitalized period in patients with dengue and other febrile illnesses

Parameter	Descriptive statistics of demographic parameters			
	Mean ± SD or %			
	Dengue		Other febrile illnesses	
Age (years)	31.3±13.4		32.0±14.2	
Male: female	61.1 %: 38.9%	3:2	64.3%: 35.7%	2:1
Average days from fever onset to admission (Days)	2.6±1.3*		3.4±1.6 *	
Hospitalization period (Days)	4.1±1.4 *		3.2±1.3 *	

P < 0.05 *, p < 0.01 **, p < 0.001 ***

Male predominance (Dengue; 61.1%, OFI; 64.3%) and highly affected young population were detected in both groups. Number of days from onset of fever to admission to the hospital is lower and hospitalization period is higher in dengue than other febrile illnesses (*Table 02*). Patients were hospitalized up to 7 days maximum. Percentage of 66% of dengue patients were discharged after 04 days from the admission. Meanwhile, 86% of patients with OFI were discharged after 04 days from the admission.

Table 03: Comparison of descriptive statistics of laboratory investigations in patients with Dengue and other febrile illnesses

Parameter	Descriptive statistics of laboratory investigations	
	Mean ± SD	
	Dengue	Other febrile illnesses
On admission		
AST U/L	114±137	112±141
ALT U/L	87±93	102±138
Total WBC count *10 ⁹/L	4.09±1.84	4.36±1.95
Absolute lymphocyte count *10 ⁹/L	0.92±0.60*	1.30±0.72*
Platelet count *10 ⁹/L	140±57	127±54
MCV	87.1±5.3 ***	85.8±4.9 ***
On discharge		
AST U/	176±150	141±136
ALT U/L	132±121	112±106
Total WBC count *10 ⁹/L	4.73±2.20 **	5.49±2.35**
Absolute lymphocyte count *10 ⁹/L	2.13±1.22**	2.72±1.49 **
Platelet count *10 ⁹/L	88±46*	115±68*
Hemoglobin male (g/dl)	14.9±1.4***	14.5±1.3***

P < 0.05 *, p < 0.01 **, p < 0.001 ***

More dengue patients have shown a high AST level than OFI on admission while ALT shows the opposite. But dengue patients had increased AST and ALT levels on discharge than OFI.

They have shown a rapid development of AST level and ALT within hospitalization period.

Both groups have shown a low total WBC count near to lower range of normal on admission. Dengue patients have shown the same lower counts on discharge while more OFI patients have reached an average total WBC count (*Table 03*). At the 3ʳᵈ day from the admission, it was indicated the lowest WBC count in dengue patients. Changing pattern in WBC within the hospitalization period is same for both groups.

Dengue and OFI have shown a lower neutrophil count near to lower limit of normal range on admission and neutropenia condition on discharge. Similar changing patterns were revealed equally. But speed in normalization of neutrophil count is slower in dengue than OFI and 3ʳᵈ day from the admission indicated the lowest neutrophil count in dengue.

When considering the lymphocyte count mutually, dengue patients have shown a lymphopenia condition on admission and it is resolved on discharge (*Table 03*). No remarkable change was happened in lymphocyte count of OFI patients on admission and discharge. Lowest lymphocyte count in dengue patients was detected in 2nd day of the admission.

Platelet count was lower than normal lower limit ($150 * 10^9$ / L) in dengue and OFI on admission and on discharge. Individually, groups have shown a thrombocytopenia condition at discharge (*Table 03*). Count of Platelet changing indicates same patterns and the lowest value during hospitalization was shown in 6th day of the admission in both groups. Mean Cell Volume (MCV), Hemoglobin (Hb), Packed Cell Volume (PCV) are not shown a significant change (*Table 03*).

Discussion

Dengue fever was very difficult to separate from other febrile illnesses which had similar characteristics and features as dengue[7,8]. Current study was based on 162 RT-PCR positive dengue patients and 126 RT-PCR negative (for dengue) patients (with other febrile illnesses) (*Table 01*). Wastage minimizing for unnecessary investigations is an essential requirement for hospitals in Sri Lankan government setting. Viral RNA was detected by RT-PCR with a high sensitivity and specificity (confirmatory test) which was used to divide suspected dengue population into 02 groups and DEN-1 serotype has shown the highest percentage from the dengue population.(*Table 01*). The study conducted in Bangladesh mentioned that DEN 2 serotype was most prevalent in their population[9].

Significant differences and similarities in demographic characters, liver transaminases and main full blood count parameters were detected between two groups of this study.

Significant differences were detected between two similarly categorized groups in a study conducted by using 111 of adult male patients[10]. In India, degree of hepatic dysfunction was detected among patients with serologically positive dengue and serologically negative OFI[5]. It seems that several studies have conducted to compare dengue from OFI. In this study, both categories of dengue patients and patients with OFI had male predominance. Male category has shown higher percentages while lower percentages were shown by female category in dengue and OFI (*Table 02*).

It was found that 82.1% of dengue patients and 79.3% of patients with OFIwere belonged to the 18-60 years (working population) age group. Current research was based on 288 suspected dengue patients. The mode of age for dengue patients was 24 and the mean value was 31.28 years. Similar findings were observed in OFI as mode of age was 22 and mean was 32 years (*Table 02*). It seems that most of the members in study population were belonged to young age group. In 2012, health statistics have shown 20-29 and 30-39 were the mostly affected age groups by dengue[2]. It was compatible with age which has shown a higher sensitivity to infection in younger. These findings were generally agreed with some studies who have found the male predominance in confirmed dengue patients[11,12,13]. As current finding, 3:2 ratios for male to female was observed in study conducted by using 425 confirmed dengue patients[14]. Significant differences were identified by analyzing average number of days from fever onset to admission and hospitalization period of patients in both groups in this study (*Table 02*). To confirm the finding, clinical history of these patients needs to be analyzed. Average of hospitalization period for dengue patients was higher in dengue. It has shown that patients with OFI were discharged earlier than dengue patients (*Table 02*). This finding was approximately correlated with a study who has observed 5.65 days as the hospitalization period[15].

The detection of changes in liver function between two groups was a main objective in current study. It was found that the mean and mode ALT and AST values on admission were increased in both dengue and OFIthan normal values. Mean, median and mode values of AST of dengue patients were higher than OFI. At discharge, average of both AST and ALT values were high in 02 populations and mean, median values were higher in dengue patients than OFI. Even though significant differences weren't detected on admission and discharge between dengue and OFI, difference between mean AST values on discharge have reached more towards to the p-value of 0.05. When comparing AST and ALT, mean value of AST in both admission and discharge were higher than mean value of ALT. Therefore, AST was believed to be taken as more prominent measurement in dengue patients on admission (early appearance) and discharge than ALT in the study (*Table 03*).

More literature has shown higher values of AST and ALT in dengue positive patients. It is confirmed that median values of AST and ALT were increased than

the normal values in dengue patients[10,16,17,18,19].

Full blood count was the main routine investigation which was performed to detect differences occurred by fever conditions. It seems that some parameters of FBC have shown significant differences between dengue and OFI. Significant lymphopenia and leucopenia were seen more in dengue patients than OFI while higher percentage of patients with OFI had neutropenia than dengue (*Table 03*).

Dengue patients have shown a lower percentage of thrombocytopenia ($< 100X10^3/mm^3$) than OFI. On discharge, average of total WBC count ($p < 0.01$), absolute lymphocyte count ($p < 0.001$) and platelet count ($p < 0.00$), were reduced significantly and mean of absolute neutrophil count was reduced in dengue than OFI (*Table 03*). Leucopenia, neutropenia, lymphopenia and thrombocytopenia were seen in more dengue patients than OFI at discharge. Considerable amount of patients in dengue had severe neutropenia. It was also found that lymphocytosis was detected in low amount of patients in dengue than OFI. Mean values of some blood parameters have shown changes in a pattern with the day of fever onset. Leucopenia was detected earlier in dengue. The lowest value of total WBC was shown in 3rd day in dengue and it was 4th day in OFI. Minimum neutrophil count (with neutropenia) was detected in 3rd day from the onset in both populations. Pattern of platelet changes was same for dengue and OFI. Therefore, platelet was not a better parameter which characterizes the dengue.It was found that the platelet count is not a better parameter to describe the severity of patients withdengue hemorrhagic fever [20].

Current findings of FBC were compatible with several previous studies. A significant difference was detected in the decreasing of mean WBC count for PCR positive patients than RT-PCR negative group[9]. It is found that significantly decreased value of total WBC as early marker in dengue patients on admission than for patients with OFI [18,21].

This research was a model study with a simple comparison of multi-parameters for adult dengue patients. Recognition of clinical symptoms on admission needs to be required for differentiation of paediatrics and adult patients with dengue and OFI. Correlation of blood parameters (PCV, MCV) with fluid management of patient is important to get an exact analysis of them. In addition to AST and ALT, total liver profile needs to be performed to validate current findings. The causative agents for OFI need to be found to differentiate from dengue.

Conclusion

RT-PCR is very suitable for early diagnosis of dengue. Lymphocyte count was most significantly changed and mean, mode and median values of AST were increased in both admission and discharge of dengue patients than Other Febrile Illnesses. Recovery of cytopenia is lower in dengue patients than OFI.

Conflict of interest

No conflict of interest occurred by the study.

Author's contribution

Data gathering and idea owner of this study	1,2
Study design	1,2,,3,4,5,6,7
Data gathering	1,2,4
Writing and submitting manuscript	1,2,7
Editing and approval of final draft	3,5,6

References:

1. Seneviratne, S L, Malavige, G N and De Silva, H J (2006): Pathogenesis of liver involvement during dengue viral infections, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, pp 608—614.

2. Annual health bulletin, (2012). Sri Lanka, Medical statistic unit, Ministry of health, pp.58-

3. Weekly epidemiological report   - A publication of the Epidemiology Unit, Ministry of health, *Nutrition and Indigenous Medicine*, 2016. **43**(43), available from http://www.epid.gov.lk/web/images/pdf/wer/2016/vol_43_no_43-english.pdf[Accessed on 18.01.2017].

4. Guidelines for treatment of dengue fever / dengue hemorrhagic fever in small hospitals. World Health Organization. Regional office for South East Asia. New Delhi, 1999, available from http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf[Accessed on 13.01.2016].

5. Bandaru, A K. Association between the degree of hepatic dysfunction and complications among serologically positive and serologically negative dengue infection in children, *Journal of Investigational Biochemistry*2015;**4**(1), pp 6-12.

6. Chung, S J, Krishnan, P U and Leo, Y S. Two cases of false-positive dengue nonstructural protein 1 (NS1) antigen in patients with hematological malignancies and a review of the literature on the use of NS1 for the detection of Dengue infection, *The American journal of tropical medicine and hygiene* 2015;**92**(2), pp 367-369.

7. Revised diagnostic testing for Zika, chikungunya and dengue viruses in US Public Health Laboratories, Centers for Disease Control and Prevention, (2016), available from https://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf[Accessed on 17. 01. 2017].

8. Chikungunya Virus, Centers for Disease Control and Prevention (2017), available from https://www.cdc.gov/chikungunya/symptoms/ [Accessed on 19.01.2017].

9. Siddiqua, M., Alam, A., Muraduzzaman, A., &Shirin, T. NS-1 antigen positive Dengue Infection and molecular characterization of Dengue Viruses in a private Medical College Hospitalin Dhaka, Bangladesh. *Bangladesh Journal of Medical Science*, 2018;**17**(4), 669-673. https://doi.org/10.3329/bjms.v17i4.38334

10. Ekanayake, D B G M, Perera, H K I, Ellepola, A N B and Athauda, S B P. Analysis of blood parameters and RT-PCR results in dengue suspected patients from Sri Lanka, *International Journal of Research in Medical and Health Sciences* 2013;**3**(1), pp 21-27.

11. Aamir, M, Masood, G and Aamir, W. Gender Difference in patients with Dengue Fever admitted in a Teaching Hospital, Lahore, *Cell*, 2014;**92 8**(1), pp12-15.

12. Shaikh, K, Memon, K N, Sarah, B, Akhtar, R, Memon, M and Memon, S. Dengue fever;an audit of risk factors among patients reporting at a tertiary care hospital in Hyderabad, *Professional Medical Journal*2014;**21**(3), pp 455-459.

13. Dhandapani, E and Sudha, M. A study of clinical profile in dengue cases, *International Journal of Pharma and Bio Sciences*2015;**6**(2), pp 84 – 92.

14. Dhir, G, Dhir, T, Suri,V, Dhir, D and Khatri, K. Hematological and Serological Test Profile in Dengue, Dengue Hemorrhagic Fever and Dengue Shock Syndrome in Bathinda Region of Punjab, *Scholars Journal of Applied Medical Sciences (SJAMS)*2015;**3**(8C), pp 2926-2930.

15. Shepard, D S, Halasa, Y A, Tyagi, B K, Adhish, S V, Nandan, D, Karthiga,K S et al Economic and Disease Burden of Dengue Illness in India, *The American journal of tropical medicine and hygiene* 2014; **91**(6), pp 1235–1242.

16. Carolinerose, J, Palaniswamy, A and Vijayarani, H. Alterations in enzyme levels in dengue patients Salem, Tamilnadu, *Journal of Global Biosciences* 2015;**4**(2), pp 14971507.

17. Itha,S, Kashyap, R, Krishnani, N, Saraswat,VA, Choudhuri,G and Aggarwal,R. Profile of liver involvement in dengue virus infection, *The national medical journal of India* 2005;**18**(3), pp 127-130.

18. Parkash, O, Almas, A, Jafri, S M W, Hamid, S, Akhtar, J and Alishah, H Severity of acute hepatitis and its outcome in patients with dengue fever in a tertiary care hospital Karachi, Pakistan (South Asia), *BMC Gastroenterology*, 2010 Available: http://www.biomedcentral.com[Accessed: 29th September 2015].

19. Jagadishkumar, K, Jain, P, Manjunath, V G and Umesh, L. Hepatic Involvement in Dengue Fever in Children, *Iranian Journal of Pediatrics*2012;**22** (2), pp 231-236.

20. Rahmasari, F., Wijayanti, D., &Khaerani, N. The Correlation Between Blood Parameters as Early Detection on Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) in Children. *Bangladesh Journal of Medical Science*, 2020; **19**(2), 273-277. https://doi.org/10.3329/bjms.v19i2.45007

21. Kalayanarooj,C S, Vaughn,D W, Nimmannitya,S, Green,S, Suntayakorn,S, Kunentrasai,N et al. Early Clinical and Laboratory Indicators of Acute Dengue Illness, *The Journal of Infectious Diseases*1997;313–321.