

A Descriptive Study of Blood Films of Patients Serologically Positive for Dengue in Hospital Tengku Ampuan Afzan, Kuantan.

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ABSTRACT

Introduction: Dengue is one of the commonest infections in Malaysia and it is a notifiable disease. Even though the diagnosis of classical dengue fever and dengue haemorrhagic fever can be recognized clinically, the diagnosis remains a challenge in areas where it could not be differentiated with other febrile illnesses. The aim of this study was to focus on the specific and consistent morphological features observed in blood films of dengue infection. **Materials and Methods:** In all 400 cases of dengue infection serologically diagnosed in the Tengku Ampuan Afzan Hospital (HTAA) during May to October 2007, only a total of 27 cases had blood films examined, and thus were included in this study. These blood films were re-examined by two pathologists from HTAA. The full blood count parameters were also retrieved and studied. **Results:** We consistently found typical reactive lymphocytes [n= 23 (85%)] and thrombocytopenia [n=21, (77.8%)] in the cases. However, leucopenia was present only in 9 cases (33%). **Conclusion:** The presence of typical reactive lymphocyte is a consistent finding in dengue fever and thus could have a significant role in supporting the diagnosis of dengue infection.

KEYWORDS: Dengue infection, reactive lymphocytes, thrombocytopenia

INTRODUCTION

Dengue virus infection is endemic in many parts of the world and widespread in tropical countries of Asia including Malaysia. Dengue virus infections are often found in the populated urban and residential areas where breeding sites for the vectors are available easily. The incidence rate of dengue virus infection in Malaysia was 181 cases per 100,000 populations in year 2007, which was above the national target of 50 cases per 100,000 population.¹ In Pahang state, incidence for dengue fever and dengue haemorrhagic fever for the year 2008 was 120 cases and 85 cases per 100,000 population, respectively.²

There are four serotypes of dengue viruses (DENV-1 through DENV-4) of the genus *Flavivirus* that are responsible for causing dengue fever (DF) and dengue haemorrhagic fever (DHF). The transmitted vectors are the *Aedes aegypti* and *Aedes albopictus*. The clinical manifestations of symptomatic dengue virus infection range from mild, undifferentiated febrile illness to classic DF or DHF. The diagnosis of dengue however

remains a challenge in areas where it could not be differentiated from other febrile illnesses.

Serological test is currently the standard diagnostic practice for confirmation of dengue infection. A positive IgM anti flavivirus antibody confirms the infection is caused by flavivirus, but the test cannot differentiate between dengue virus and other flavivirus infection. The commercial Anti-dengue virus immunoglobulin M kits had test sensitivities of 21% to 99% with test specificities of 77% to 98%.³ This explains the expected false positivity of current dengue serology test.⁴

The aim of this study was to look at the consistent morphological features seen in peripheral blood films of patients with dengue that can support the diagnosis of the disease in addition to the serological test. The full blood count parameters were also assessed in this study.

MATERIALS AND METHODS

This retrospective study was carried out in Tengku Ampuan Afzan Hospital (HTAA), Kuantan, Pahang. Particulars of all patients who had positive serology results for dengue infection (IgG and/or IgM Panbio™ Dengue Capture ELISA, Aus) from May 2007 to October 2007 were retrieved from records. Cases that had full blood pictures (full blood count and blood film) done during the illness were selected for this study.

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Out of 400 patients who had positive serology results for dengue infection during the 5 months period, only 27 patients had full blood picture analysis performed. These slides were then retrieved and reviewed by two pathologists. Morphology of all blood cell series was studied. Herein, a typical reactive lymphocyte is defined as large lymphocyte with moderate nuclear cytoplasmic ratio and intensely basophilic cytoplasm.⁵ Among the blood parameters reviewed were: platelet count, haematocrit value and the differential white cell count. Thrombocytopenia was considered when the platelet count is less than $140 \times 10^9/L$. Other blood parameters were defined based on the standard reference range practices by HTAA (Refer appendix).⁶

RESULTS

In total there were 400 patients who had positive serology results for dengue during the period of 5 months. Of these only 27 had full blood picture performed and reviewed in this study. Majority of the full blood picture studied belonged to adults [n=25 (92.6%)]. Malays constituted more than 70% (n=21) of the cases. There were almost equal number of patients diagnosed with either IgM positive [n= 12 (44.5%)] or both IgG and IgM positive [n= 15 (55.5%)] (Table I). The observation of a large lymphocyte with very basophilic cytoplasm fulfilled the criteria set for typical reactive lymphocyte (Figure 1). This typical reactive lymphocyte was observed in 23 out of 27 of the studied cases (85.1%). Thrombocytopenia and leucopaenia were present in 21 (77.8%) and 9 cases (33.3%) respectively. (Table II). Majority of case studied [n=21 (77.8%)] had normal haematocrit levels.

Table I. Demographic data of the 27 studied sample.

Characteristics	n	%
Age group (years)		
1-12	2	7.4
13-39	21	77.8
40-59	4	14.8
Sex		
Female	15	55.5
Male	12	44.5
Race		
Malays	21	77.8
Chinese	2	7.4
Indians	2	7.4
Others	2	7.4
Dengue serology		
IgM	12	44.5
IgG and IgM	15	55.5

Table II. Haematological findings of the studied cases (n=27).

Variables	Findings	n	%
Haemoglobin levels	Normal	16	59.2
	Anaemia	11	40.7
Haematocrit	High	1	3.7
	Normal	21	77.8
	Low	5	18.5
Total leucocyte count	Leucocytosis	5	18.5
	Normal	13	48.1
	Leucopenia	9	33.3
Platelets	Normal	6	22.2
	Thrombocytopenia	21	77.8
Neutrophils	Neutrophilia	1	3.7
	Normal	17	62.9
	Neutropenia	9	33.3
Lymphocytes	Normal	23	85.1
	Lymphopenia	4	14.8
Monocytes	Monocytosis	4	14.8
Typical reactive lymphocytes		23	85.1
Dysplastic changes		4	14.8

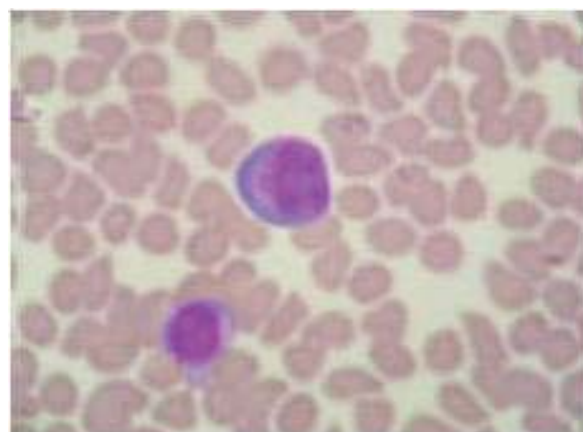


Figure 1. Typical reactive lymphocyte

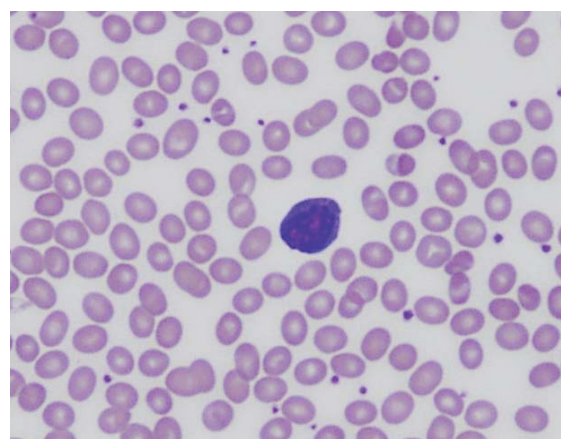


Figure 2. The image shows a typical reactive lymphocyte which is described as a large lymphocyte with moderate nuclear cytoplasmic ratio and intensely basophilic cytoplasm. (The blood film is stained with Wright's stain and taken at 100x magnification)

DISCUSSION

Full blood film examination is not a routine laboratory test requested in the investigation of dengue. Thus

there were only 27 cases (7%) out of 400 confirmed dengue cases that had their blood films done. The reasons for requesting a blood picture investigation in these 27 cases could not be validated as it was not clearly stated in the request forms.

Thrombocytopenia is a typical finding in dengue haemorrhagic fever.⁷ Dietz et al reported that thrombocytopenia was present in 34% of patients with dengue fever.⁸ In our study 77.8% (21) of the patients had thrombocytopenia. Wichman et al showed that in addition to thrombocytopenia, patients with confirmed dengue infection also had leucopenia.⁴ This was infrequent finding in cases with false positive dengue serology.

Contrary to Carlos et al's findings, haemoconcentration is not a feature in our study subject.⁹ The reason for this is because haemoconcentration usually occurs in patients with dengue shock syndrome. Furthermore, the time of sampling in relation to the course of the illness was not documented in this study. This has contributed to the discrepancy.

Although, the presence of atypical lymphocytosis was highlighted in several studies, none of the studies described the morphology of the lymphocytes.^{9,10} Our finding showed that the "typical" reactive lymphocyte was consistently seen in the majority of patients.

CONCLUSION

Based on our findings, a full blood picture can have a significant role in supporting the diagnosis of dengue. It can complement the clinical picture and serological diagnosis of dengue especially in cases where the clinical presentations are atypical.

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REFERENCES

1. Annual Report 2007. Vector Borne Diseases Section, Ministry of Health, Malaysia, (Unpublished).
2. Annual Report 2008. Vector Borne Diseases Unit, Department of Health, Pahang, Malaysia. (Unpublished)
3. Hunsperger EA, Yoksan S, Buchy P, et al. Evaluation of commercially available Anti-Dengue Virus Immunoglobulin M test. *Emerg Infect Dis* 2009; 15(3):436-40
4. Wichmann O, Stark K, Shu PY, et al. Clinical features and pitfalls in the laboratory diagnosis of dengue in travellers. *BMC Infect Dis* 2006; 6:120
5. Bell A, Sallah S. *The Morphology of Human Blood Cells*, 7th Ed. 2005; Abbot, US.

6. Handbook for pathology services, Hospital Tengku Ampuan Afzan. Ver. 2007.
7. World Health Organization. Clinical and laboratory guidelines for dengue fever and dengue haemorrhagic fever/dengue shock syndrome for health care providers.
8. Dietz V, Gubler DJ, Ortiz S, et al. The 1986 dengue and dengue haemorrhagic fever epidemic in Puerto Rico: epidemiologic and clinical observations. *P R Health Science J* 1996; 15:201-10
9. Carlos CC, Oishi K, Cinco MT, et al. Comparison of clinical features and haematologic abnormalities between dengue fever and dengue haemorrhagic fever among children in the Philippines. *Am J Trop Med Hyg* 2005; 73(2):435-40
10. Gubler DJ. Dengue and dengue haemorrhagic fever. *Clin Microbiol Rev* 1998; 11(3):480-96

Appendix

Reference range for haematology parameters used in this study.

Parameters	Reference range	Unit
Haemoglobin	11.0 - 14.0	g/dL
Haematocrit	4.00 - 5.20	10 ¹² /L
Total white cell count Neutrophils	5.0 - 15.0	10 ⁹ /L
Lymphocytes	45.0 - 75.0	%
Monocytes	24.0 - 45.0	%
Platelets	2.0 - 10.0	%
	150 - 400	10 ⁹ /L

