The importance of monitoring haematologic changes, thrombocytopenia and monocytosis in dengue haemorrhagic fever

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Abstract

Background. Dengue fever is one of the most serious consequences of mosquito-borne infection worldwide. Haematologic derangement with increased activated monocytes and thrombocytopenia and endothelial activation may be responsible for plasma leakage and shock. The aim of the study was to investigate the haemostatic changes, thrombocytopenia and monocytosis in dengue haemorrhgic fever.

Materials and Methods. Eighty patients (male n=40) were recruited at day 3 of febrile state. Their ages ranged between 5 to 73 years. Blood sampling for Full Blood Count was performed daily till discharge from hospital when their temperature returns to normal and platelets >50 $x10^9$/L. Haemoglobin, haematocrit, white blood cells, platelets and monocytes were determined.

Results and Discussion. 28 patients were below 16 years old and the parameters studied were compared to the other 52 patients. No statistical significant parameters were seen between the two groups and their data were merged and analysed separately, grades I (n=46) and II/III DHF (n=34). Haemoglobin and haematocrit levels declined following hydration treatment. Improvement in white blood cell count and platelets was seen in Grade I with further elevated monocytes from day 5. Thrombocytopenia was still observed by day 7 in Grade II/III with greater further increase in monocytes. Overall Monocytosis was 52.5% at admission and 90,5% by day 7.

Conclusion. Haemostatic changes with thrombocytopenia and monocytosis in severe DHF Grade II/III suggesting on-going inflammation or plasma leakage. It may not be ideal to discharge patients when their temperature returns to normal and platelets >50 $x10^9$/L. Monocyte profile is therefore suggested.

Keywords. DHF, thrombocytopenia, monocytosis

Introduction

Dengue fever is one of the most serious consequences of mosquito-borne infection worldwide. The disease re-emerged as a major public health problem in more than 100 countries in the tropical and sub-tropical climate zones (1), The number of cases reported increased from 2.2 million in 2010 to 3.2 million in 2015 (2). Dengue fever is a severe, flu-like illness that affects children and adults but seldom causes death, even though there is no specific treatment for it. Dengue haemorrhage fever (DHF) has been defined by the World Health Organisation (3) as dengue with and without warning signs of plasma leakage and has been defined into four grades. Grade I, with warning signs of plasma leakage but no bleeding, grade II, dengue with warning signs of plasma leakage and bleeding. Grade III, severe dengue with threatened shock, grade IV, severe dengue with profound shock, grades III and IV are known as dengue shock syndrome (DSS). Patients who did not fulfil the WHO criteria of DHF could otherwise be considered as having non-severe form of the disease (4). The diagnosis of dengue virus infection should be confirmed by viral isolation, dengue non-structural protein-1 (NS1) antigen, and/or dengue specific IgG or IgM antibodies. Endothelial damage may be caused by the virus itself, cytokine secretion from monocytes/macrophages and/or complement activation (4). Endothelial activation may be responsible for plasma leakage and shock (5). The monocyte responds to inflammation and chemotactic stimuli where it can mature into the macrophage with greater phagocytic activity and increased content of hydrolytic enzymes. Phagocytic immune cells play a critical role in restriction of viral dissemination and in thrombocytopenia in DF have been suggested (6). Monocytosis is associated with a variety of diseases related to the body's immune system (7). Haemostatic changes occur in the early stage of illness in all severity of dengue (8). Haematological derangement in DHF showed high haematocrit (Hct), low white blood cell (WBC) count, low neutrophils, high lymphocytes and increased atypical monocytes with low platelets. These returned to normal state during the convalescent stage (4). Plasma leakage cause haemoconcentration with elevated haemoglobin (Hb) but bleeding induced anaemia. Thrombocytopenia is commonly observed in both mild and severe dengue syndrome and associated with clinical outcome (3, 9, 10). Thrombocytopenia usually occurs between days 3 and 7 of fever and returns to normal by the 8th day of the convalescent stage (8). Platelet consumption caused by high levels of platelet activating factor released from monocytes and activated platelets may be engulfed by transient surge on monocytes contributing to thrombocytopenia was reported (10-13). Dysfunctional platelets in conjunction with transiently activated monocytosis could be a key event in the pathogenesis of developing DHF after dengue infection (6). Studies from Venezuela reported monocytosis in 68.8% of their cases (14) and 75% in Chennai, India (15). Inverse correlation between monocytes and platelets are seen in severe dengue and during recovery it was again observed monocytes were reduced with corresponding increase in platelets. In DF without complication this association was not seen suggesting that the association can be taken as a marker to predict severe dengue and proposed that monocytosis

with thrombocytopenia can be used as a marker for subsequent development of severe dengue (15).

The aim of the study was to investigate the haematologic changes, thrombocytopenia and monocytosis in dengue haemorrhagic fever.

Materials and Methods

The study received ethical approval from the Health Research Ethical Committee (No 571/TGI/KEPK FK USU RSUP HAM/2017), Medical Faculty, Universitas Sumatera Utara/Haj Adam Malik Hospital, Medan, Indonesia.

Subjects. Every patient with fever visiting the Accident and Emergency (A/E) Clinic were approached to take part in the study. Inclusion criteria: Patients who have fever of $\geq 38.5^{o}$ C for at least 3 days either with muscle pain/bone pain were recruited when they visited the A/E Clinic of the hospital. Patient Informed Consent was obtained and in children parental consent was obtained before they are recruited in to the study. Blood sampling and stat laboratory tests were performed. Patients with positive for either NS1 antigen, anti-DHF IgG or IgM were recruited. Exclusion criteria: patients who were negative to either, NS1, anti-DHF IgG or IgM and who showed evidence of bacterial infection and non-consent was excluded. The patients when admitted to the hospital were febrile for three days and thus when recruited, as day 3 of febrile state. Eighty patients who fulfilled the Inclusion Criteria were recruited and their ages ranged between 5 years to 73 years old (mean 23.9 ± 14.6 years). Treatment with normal saline infusion were given to each patient and when diagnosis of dengue haemorrhage fever was confirmed hydration is continued with addition of potassium 25 mEq for every litre of normal saline. Anti-pyretic paracetamol was given orally if fever persist. The treatment is continued until patient is discharged when the temperature returned to normal and platelets >50 x10^9/L as set out in the hospital's protocol. This occurs in either Day 5, day 6 or day 7 from febrile state except for one male patient Grade III who opted for further treatment in a neighbouring country after day 7 in the hospital. He had platelets of 15 x 10^9/L and monocytes 25%.

Blood sampling. About 8 mL blood from a clean venepuncture was obtained and drawn into 3.0 mL and 5 mL EDTA vacutainer tubes for Full Blood Count (FBC) and NS1 antigen and anti-DHF IgG/IgM. Blood sampling for FBC was performed daily until patient is discharged.

Laboratory analysis. Haemoglobin (Hb), haematocrit (Hct), white blood cells (WBC), platelets and monocytes were determined in the Haematology Analyzer Sysmex XN100 (Sysmex Corporation, Kobe, Japan) and immunochromatography (ICT/Rapid test) for plasma NS1 antigen and anti-DHF IgG/IgM.

Statistical analysis. The Statistical Package for Social Sciences (SPSS 22 IBM Corp) was used to perform statistical analysis. The group mean independent sample t-test for differences between the study periods and between dengue Grade I and Grade II/III were analysed. Analysis of Variance (ANOVA) for the parameters studied and Pearson's correlation was also determined. A P-value of <0.05 was considered statistically significan

Results

Clinical characteristics in dengue haemorrhagic fever patients at the Accident and Emergency Clinic and admission to the hospital at day 3 of fever and the duration of study period before discharge from hospital.

Eighty patients (male n = 40, female n= 40) who fulfilled the inclusion criteria and gave written informed consent were recruited. Their ages ranged between 5 years and 73 years old (mean 23.9 ± 14.6 years). Twenty-eight of the patients were below 16 years old (mean $10.5 ± 3.2$ years) and the rest (n=52, mean $31.0 ± 13.3$ years). Clinical characteristics and grading of dengue haemorrhagic fever patients after their laboratory tests were positive for either NS1 antigen and ant-DHF IgG/IgM (grade I n=46, grade II n=29, grade III n=5). and the duration of study between day 3 to day 7 are shown in Table 1. One of the patient with severe DHF Grade 3 opted for further treatment in the neighbouring country after day 7 in hospital. He had severe thrombocytopenia with platelets of 15 x10^9/L and monocytes 25% when discharged.

Haematological studies (mean ± SD) in dengue haemorrhagic fever comparison on day 3 of admission and between days 4 to 7 of study in Grade I, Grade II/III and between Grade I and II/III on day 3 to day 7. Analysis of Variance (ANOVA) was also determined. (Table 2)

Twenty-eight of the patients were below 16 years old had their haematologic parameters studied compared to the other older patients (n=52). Besides their ages, there were no statistically significant results seen between the two groups of cohorts. Therefore, their data were merged and analysed separately between the grades of DHF (Grade I, n=46, Grade II/III n=34).
Grade I. There were no statistical differences in parameters studied on admission (day 3) compared to day 4, 5 ,6 and day 7 except for a significant increase in monocytes to mean 10.8 ± 3.7% (P=0.03) on day 5 from febrile state. Haemoglobin and haematocrit showed a declining mean trend but they did not reach statistical significance, similarly for white blood cell count and platelets which showed an increased mean trend. Monocytes showed mild increase (mean 9.2 ± 4.2) from normal of 8% at admission, significant increase at day 5 mean 10.8 ± 3,7 % (P=0,03) was seen with no significant differences at day 6 or day 7 despite the increased mean levels observed. ANOVA was not statistically significant for the parameters studied.
Grade II/III. Haemoglobin and haematocrit showed a significant mean decline by day 6 from day 3 (P= 0.03, P=0,04) respectively. White blood cells and platelets did not show significant differences from at admission, but mean platelet numbers remain below 100 x 10^9/L except for an improvement on day 6 but it was not significantly different from day 3, largely due to the wide variation in platelets seen. Thrombocytopenia persist after hospital discharge by day 7. However, monocytes showed a significant increase from day 4 (P=0,03), day 5 (P=<0.001), day 6 *(P*= 0.001) and in the thirteen patients in the study at day 7 (P=0.02), ANOVA showed only significant differences in Monocytes *(P*=0.001) during the study period, Table 2.
Grade I vs Grade II/III. Significantly decreased platelets was seen in Grade II/III DHF compared with Grade I DHF between day 3 on admission (P=0.01), day 4 (P=0.01) and day 5 (P=0.02), Monocytes showed a significant increase in Grade II/III DHF compared with Grade I on days 4, 5, 6 (P=<0.001) and 7 (P= 0.01) Table 2..

Monocytosis in dengue haemorrhagic fever, at admission (day 3) and discharge after day 5, day 6 and day 7 (Table 3).

Grade 1. Monocytosis (monocytes >8%) was seen in 50.0% (23/46) of patients on admission and when discharged from hospital at day 5 it was 34.8% (16/46), day 6, 35.0% (7/20), and day 7, 75.0% (6/8).

Grade II/III. Monocytes at admission was 55.9% (19/34) and when discharged from hospital at day 5, 32.3% (11/34), day 6, 31.5% (7/22) and day 7, 100% (13/13).

Mean monocytes numbers from admission to discharge for Grade I and Grade II/III DHF during the study period is shown in Figure 1.

Correlation studies. Pearson's correlation showed significant correlation between haemoglobin and haematocrit (P=<0.001) and a negative correlation between monocytes and platelets (P=0.002) in both Grades I and II/III DHF.

Discussion

Dengue fever is one of the most serious consequences of mosquito-borne infection worldwide. Haematologic derangement are seen in DHF with increased atypical monocytes and thrombocytopenia (4). Endothelial activation may be responsible for plasma leakage and shock (5), it causes plasma concentration with elevated haemoglobin and thrombocytopenia is usually present in mild and severe dengue syndrome (3, 9, 10). Platelets may be engulfed by activated monocytes contributing to thrombocytopenia and monocytosis with thrombocytopenia may be used as marker for subsequent development of severe dengue (6, 15). Monocytosis have been reported to be present in 68.8% of cases in Venezuela (14) and 75% in Chennai, India (15).

In our study DHF patients' haematocrit and haemoglobin levels declined following hydration treatment especially by day 6 in Grade II/III patients. Improvement in white blood cell count and platelets by day 7 was seen in Grade I DHF with further elevated monocytes from day 5. In Grade II/III DHF thrombocytopenia was still observed on day of discharge at day 7. Platelets were significantly lower with monocytes increased much more than seen in Grade I DHF. Significantly increased monocytes were seen after admission and at discharge at day 7 in DHF. Significant correlation between haemoglobin and haematocrit was seen in DHF with negative correlation between platelets and monocytes which was similarly observed by Sivathanu and co-workers (15). ANOVA showed monocytosis was evident in Grade II/III DHF compared to mild monocytosis in Grade I DHF. Monocytosis was seen at day 3 of febrile state at admission was observed in 50.0% in Grade I and 55.9% in Grade II/III DHS patients (overall 52.5%). At discharge from hospital at day 7, monocytosis was further elevated to 75.0% in Grade I and 100% in Grade II/III DHF (overall 90.5%). The incidence of monocytosis differs from that reported (14, 15). The haemoglobin/haematocrit levels at admission suggest haemoconcentration in DHF, thrombocytopenia and monocytosis suggest endothelial activation which concern plasma leakage. Monocytosis remains elevated by day 7 suggesting that inflammation or endothelial activation is still present when patients were discharged when their temperature returns to normal with

platelets >50 x10^9/L may not be ideal. Monocyte profile may be crucial before discharge is therefore suggested. Follow-up of patients following discharge was not performed.

Conclusion. Haematologic changes was seen in DHF with thrombocytopenia and monocytosis more evident in severe DHF Grade II/III suggesting on-going inflammation or plasma leakage. It may not be ideal to discharge patients when their temperature returns to normal and platelets >50 x10^9/L. Monocyte profile is therefore suggested.

Acknowledgement

Conflict of Interest

The authors declared that they have no conflict of interest.

References

1. World Health Organisation. Global strategy in dengue prevention and control 2012-2020. Geneva, WHO 2012

2. World Health Organisation. Dengue and severe dengue. WHO Fact sheet updated April 2017.

3. World Health Organisation Dengue guidelines for diagnosis, treatment, prevention and control. New ed, Geneve, Switzerland, World Health Organisation 2009.

4. Chuansumrit A and Chaiyaratana W. Hemostatic derangement in dengue haemorrhagic fever. Thromb Res 2014; 133:10-16.

5. De Castro RA, De Castro JA, Barez MY, Frias MV, Dixit J, Geneteux M. Thrombocytopenia associated with dengue hemorrhagic fever responds to intravenous administration of anti-D (Rh(O)-D) immune globulin. Am J Trop Med Hyg 2007; 76:737-42.

6. Tsai JJ, Chang S, Chang K et al. Transient monocytes sugates low platelet count in adult dengue patients. Biomed Hub 2017; 2:457785; doi:10.1159/000457785.

7. ShweSin, Win LL, Aung TY,TheinMuanguang, Kay KhaingSwe. Approach to the patients with monocytosis. IOSR J Dental Med Sciences (IORS-JDMS) 2015; 114 (5): ver II:81-6.

8. Mourao MP, Lacerda MV, Macedo VO, Santos JB. Thrombocytopenia in patients with dengue virus infection in the Brazilian Amazon. Platelets 2007; 18:605-12.

9. Honda S, Saito M, Dimano EM et al. Increased of phagocytosis of platelets from patients with secondary dengue virus infection by human macrophages. Am J Trop Med Hyg 2009; 80:841-5.

10. Sosothukul D et al. Hemostatic studies in dengue fever. SEA J Trop Med Public Health 2015; 46: suppl 1:43-5

11. Yang KD, Lee CS, Shalo MF. A higher production of platelet activating factor in ex vivo heterologous secondary dengue-2 virus infection. Acta Microbiol Immunology Hung 1995; 42:403-7.

12. Tsai JJ, Jen YH, Chang JS, Hsiao HM, Noisakran S, Perng GC. Frequency alterations in key innate immune cell components in the peripheral blood of dengue patients detected by FACS analysis. J Innate Immunol 2011; 3:530-40.

13. Wan SW, Yang YW, Chu YT et al. Anti-dengue nonstructurl protein 1 antobodies contribute to platelet phagocytosis by macrophages. Thromb Haemost 206; 115:646-56.

14. Villegas E, Villegas N, Morales S et al. Occurrence of neutrophil left shift in dengue haemorrhage fever. Rev Soc Med 2003; 34(1):6-8.

15. Sivathanu S, Manickam K, Sampath S et al. To study the clinical profile of dengue fever and evolve a prognostic marker based on hematological parameters for severe dengue. Int J Contemporary Pediatrics 2017; 4(2):538-42.

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Table 1. Clinical characteristics in dengue haemorrhagic fever patients at the Accident & Emergency Clinic and admission to the hospital at day 3 of fever and duration of study period before discharge from hospital.

Dengue fever grades	I	II	III
Fever only	36	9	0
Fever/skin rash	10	9	0
Fever/skin rash/short of breath	0	1	0
Fever/skin rash/short of breath/edema/ascites	0	10	4
Fever/skin rash/short of breath/edema/ascites/shock	0	0	1
Total:	46	29	5
Duration of Study:			
Day 3 to Day 5	25	10	1
Day 3 to Day 6	13	9	1
Day 3 to Day 7	8	10	3
Total:	46	29	5

Table 2. Haematological studies (mean ± SD) in dengue haemorrhagic fever comparison on day 3 of admission and between days 4 to 7 of study in Grade I, Grade II/III and between Grade I and II/III on day 3 to day 7. Analysis of Variance (ANOVA) was also determined.

Grade I	Hb g/dL	Hct (%)	WBC /mm^3	Plats (x10^9/L)	Mono (%)
Day 3 (n=46)	13.7 (1.5)	40.5 (4.7)	5499 (3948)	117.9 (73.1)	9.1(4.2)
Day 4 (n=46)	13.3 (1.5)	39.4 (4.6)	5958 (4639)	95.5 (62.1)	9.4 (3.9)
P	0.25	0.25	0.61	0.12	0.68
Day 5 (n=46)	13.1 (1.5)	39.0 (4.7)	6109 (4062)	102.4 (71.4)	10.8 (3.7)
P	0.07	0.14	0.47	0.33	**0.03**
Day 6 (n=20)	12.3 (2.0)	38.9 (5.7)	7894 (6664)	112.8 (95.8)	10.5 (3.9)
P	0.25	0.27	0.15	0.83	0.20
Day 7 (n=8)	11.9 (1.9)	36.3 (6.6)	8784 (3973)	131.5 (114.1)	11.0 (2.9)
P	0.09	0.08	0.06	0.75	0.13
ANOVA *P*	0.14	0.11	0,17	0.54	0.17
Grade II/III					
Day 3 (n=34)	14.1 (1.6)	41.1 (3.8)	5290 (4124)	78.2 (63.9)	10.8 (6.8)
Day 4 (n=34)	13.8 (1.8)	40.4 (4.7)	4947 (2804)	63.1 (48.8)	14.6 (7.2)
P	0.51	0.49	0.69	0.28	**0.03**
Day 5 (n=34)	13.5 (1.5)	39.3 (5.3)	5374 (2358)	71.5 (51.2)	17.5 (6.8)
P	0.13	0.10	0.92	0.64	**<0.001**
Day 6 (n=22)	13.3 (1.5)	38.6 (5.2)	4879 (1613)	118.0 (108.8)	17.7 (6.9)
P	**0.03**	**0.04**	0.42	0.13	**0.001**
Day 7 (n=13)	13.5 (1.3)	39.3 (4.6)	6292 (1643)	95.2 (51.4)	15.7 (5.8)
P	0.25	0.22	0.24	0.35	**0.02**
ANOVA *P*	0.36	0.30	0.73	0.16	**0.001**
Grade I vs Grade II/III					
Day 3 *P*	0.23	0.53	0.82	**0.01**	0.20

Day 4 P	0.17	0.36	0.23	**0.01**	**<0.001**
Day 5 P	0.21	0.82	0.31	**0.02**	**<0.001**
Day 6 P	0.68	0.89	0.06	0.76	**<0.001**
Day 7 P	0.42	0.74	0.05	0.34	**0.01**

Table 3. Monocytosis in dengue haemorrhagic fever, at admission (day 3) and discharged after day 5, day 6 and day 7.

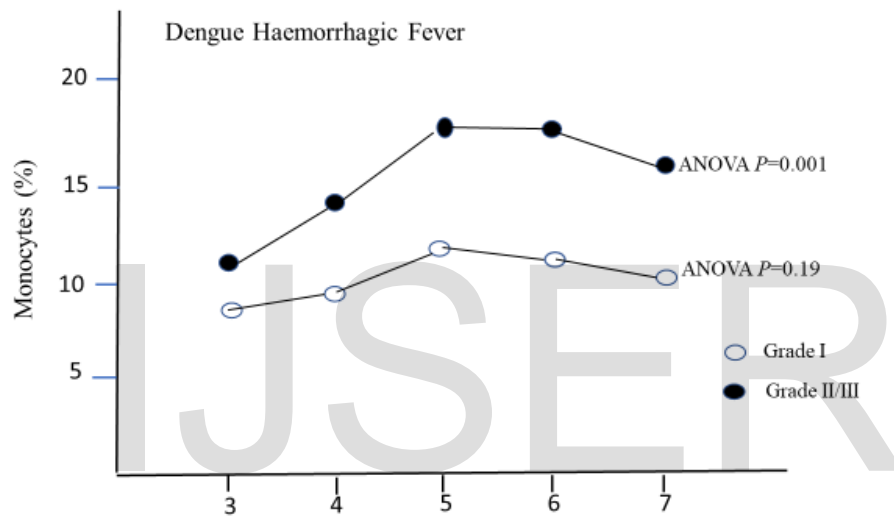|	Grade I	Grade II/III	Overall
At admission day3 (3rd day from febrile state)	50.0% (23/46)	55.9% (19/34)	52.5% (42/80)
Day of hospital discharge:			
Day 5	34.8% (16/46)	32.3% (11/34)	33.8% (27/80)
Day 6	35.0% (7/20)	31.5% (7/22)	33.3% (14/42)
Day 7	75.0% (6/8)	100.0% (13/13)	90.5% (19/21)

Figure 1. Monocytosis in Grade I and Grades II/III dengue haemorrhagic
fever day 3 to day 7 from febrile state.