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CASE REPORT

Haemophagocytic Lymphohistiocytosis (HLH) in Adult with Dengue Infection

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ABSTRAK

'Haemophagocytic lymphohistiocytosis' (HLH) atau 'haemophagocytic syndrome' adalah sindrom yang jarang berlaku tetapi mempunyai risiko mengancam nyawa. Sindrom ini melibatkan pengaktifan imunisasi yang berlebihan dengan tanda-tanda klinikal yang tidak spesifik. HLH adalah salah satu komplikasi jangkitan denggi. Seorang wanita berusia 69 tahun dirawat untuk denggi dengan disfungsi organ-organ badan dan jangkitan paru-paru yang memerlukan bantuan alat pernafasan. Walaupun pemindahan darah telah dilakukan, sitopenia yang berterusan dan tiada tanda pendarahan menimbulkan kecurigaan kehadiran HLH. Ujian darah yang lebih lanjut menunjukkan keadaan hipertrigliseridemia, hipofibrinogenemia dan hiperferritinemia. Aspirasi sumsum tulang menunjukkan 'haemophagocytosis'. Pesakit memenuhi kriteria diagnostik untuk HLH oleh HLH-2004. Keadaan pesakit bertambah baik selepas diberi imunoglobulin intravena (IVIG) dan dexamethasone intravena dalam dos yang semakin berkurangan. Rawatan spesifik awal untuk HLH dengan IVIG dan/atau kortikosteroid adalah penting tetapi diagnosa biasanya ditangguhkan kerana tanda-tanda klinikal dan ujian makmal yang tidak spesifik. Indeks kecurigaan yang tinggi dengan bantuan kriteria diagnostik oleh HLH-2004 dan HScore membantu mengenali sindrom ini.

Kata kunci: denggi, 'haemophagocytic lymphohistiocytosis', 'haemophagocytic syndrome'

ABSTRACT

Haemophagocytic lymphohistiocytosis (HLH) or haemophagocytic syndrome is a rare but life-threatening syndrome of excessive immune activation with nonspecific

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clinical presentation. HLH is one of the complications in dengue infection. A 69-year-old lady was treated for severe dengue with multi-organ dysfunction with superimposed pneumonia, requiring mechanical ventilation. However, persistent cytopenia despite blood transfusion without evidence of haemorrhage raised the suspicion of HLH. Further blood investigations revealed hypertriglyceridaemia, hypofibrinogenaemia and hyperferritinaemia. Bone marrow aspiration showed haemophagocytosis. Patient fulfilled the diagnostic criteria for HLH by HLH-2004 trial. Her HScore is 281, with the probability of having HLH is 99.9%. Patient's condition improved after administration of intravenous immunoglobulin (IVIG) and intravenous dexamethasone in tapering doses. Early specific treatment of HLH with IVIG and/or corticosteroid is important but diagnosis is usually delayed due to nonspecific clinical findings and laboratory results. High index of suspicion with the aid of diagnostic criteria by HLH-2004 trial and HScore is helpful to recognise this syndrome.

Keywords: denggue, haemophagocytic lymphohistiocytosis, haemophagocytic syndrome

INTRODUCTION

Dengue infection is a common arthropod-borne viral disease in tropical countries, with the incidence rate of 361 cases per 100,000 populations in 2014 among Malaysian (Ministry of Health Malaysia 2015). The prevalence of dengue infection among patients presenting with non-specific viral fever in the Primary Care Centre of Hospital Universiti Kebangsaan Malaysia (HUKM) and Batu 9 Health Clinic Hulu Langat was 32.9% (Tong et al. 2006).

Haemophagocytic lymphohistiocytosis (HLH) or haemophagocytic syndrome (HPS) is a rare but life-threatening syndrome of excessive immune activation leading to cytokine storm. Primary HLH, also known as familial haemophagocytic lymphohistiocytosis , is caused by gene mutation that produced a dysregulated immune activity. Secondary or acquired HLH is described as HLH in patient without gene mutation and triggered by an event (viral infection, autoimmune disease, rheumatological disease, malignancy).

Existing reports showed that HLH may develop in both primary and secondary dengue (Wan Jamaludin et al. 2015; Tan et al. 2012). This syndrome most commonly affects the paediatric population but rarely in adults. It is a potentially fatal complication of dengue fever, which is usually under-recognised and under-reported in Malaysia. Routine usage of diagnostic criteria for HLH via HLH-2004 trial (Table 1) (Henter et al. 2007) and Hscore (Fardet et al. 2014) in dengue patient with multi-organ dysfunction (MOD) will allow early recognition of HLH. Early initiation of specific

Table 1: Diagnostic criteria for HLH adopted from HLH-2004 trial (Henter et al. 2007)

The diagnosis HLH can be established if either A or B below is fulfilled	
A. Molecular diagnosis consistent with HLH	Pathologic mutations of PRF1, UNC13D, Munc18-2, Rab27A, STX11, SH2D1A, or BIRC4.
B. Five of the 8 criteria listed below are fulfilled:	
1. Fever	≥ 38.5°C
2. Splenomegaly	
3. Cytopenias (affecting at least 2 of 3 lineages in peripheral blood)	Haemoglobin <9 g/dL, platelets <100 × 10^9/L, neutrophils <1 × 10^9/L
4. Hypertriglyceridaemia or hypofibrinogenaemia	Fasting triglyceride >3 mmol/L or fibrinogen <1.5 g/L
5. Haemophagocytosis in bone marrow, spleen, lymph nodes, or liver	
6. Low or absent NK-cell activity	
7. Ferritin	>500 µg/L
8. Elevated soluble CD25 (α-chain of sIL2r)	>2,400 U/ml

Abbrevation: BIRC4=baculoviral IAP repeat-containing protein 4; CD=cluster of differentiation; HLH=haemophagocytic lymphohistiocytosis; Munc18-2=mammalian uncoordinated-18 proteins; NK=natural killer; PFR1=perforin 1; Rab27a=Ras-related protein Rab-27A; SH2D1A=SH2 domain–containing protein 1A; sIL2r=soluble interleukin-2 receptor; STX11=Syntaxin 11; UNC13D=protein unc-13 homolog D.

treatment for HLH may improve clinical outcome.

CASE REPORT

A 69-year-old woman with underlying dyslipidaemia and major depressive disorder, presented to the Emergency Department (ED) with fever for three days, associated with giddiness for four days and generalised body weakness and loss of appetite for one day. Her family history was unremarkable. On arrival to ED she was alert, normotensive with the blood pressure of 124/78 mmHg, heart rate of 122 bpm, and temperature of 39.5°C. Her oxygen saturation was 91% under room air. Examination of the chest and abdomen revealed crepitation over bilateral lower zone with no hepatosplenomegaly. Neurological examination was unremarkable.

Her initial full blood counts showed white blood cells (WBC) 5.3 x 10^9/L, haemoglobin 13.5 g/dL, haematocrit 39.5%, platelet 134 x 10^9/L. Chest X-ray showed left lower zone and bilateral perihilar opacities. Arterial blood gas showed type one respiratory failure with pH 7.495, $PaCO_2$ 3.37 kPa, PaO_2 7.49 kPa, bicarbonate 22.4 mmol/L, base excess -3.4. Patient initially was treated for community-acquired pneumonia with type one respiratory failure. Intravenous (IV) amoxicillin-clavulanate and nasal prong oxygen 3 L/min was given followed by admission into the general ward.

Dengue serology was sent on day 2 (D2) of hospitalisation as the repeated WBC dropped to 2.2 x 10^9/L and platelet count dropped to 86 x 10^9/L. Both dengue IgM and IgG were positive. Patient was treated for severe dengue and MOD, namely acute kidney failure with urea 23 mmol/L and creatinine 527.7 µmol/L,

requiring intermittent haemodialysis; and deranged liver function test with raised alanine aminotransferase (ALT) (436 U/L) as well as raised aspartate aminotransferase (AST) (1526 U/L). On D4 of hospitalisation, as her respiratory distress was worsening, the patient was started on non-invasive ventilation (NIV) and transferred to intensive care unit (ICU). Repeated chest X-ray showed worsening opacities in left lower zone to midzone with bilateral pleural effusion. Patient was empirically treated for superimposed community acquired pneumonia and antibiotic was changed to IV piperacillin-tazobactam.

On D7 of hospitalisation, her respiratory distress worsened despite being on NIV and required higher FiO_2. Patient was intubated. Post-intubation chest X-ray showed similar changes. In view of increasing procalcitonin level from 1.78 ng/ml to 5.99 ng/ml (reference range <0.05 ng/ml), antibiotic was escalated to IV meropenem and was given for total of seven days. Otherwise, serial blood cultures showed no growth.

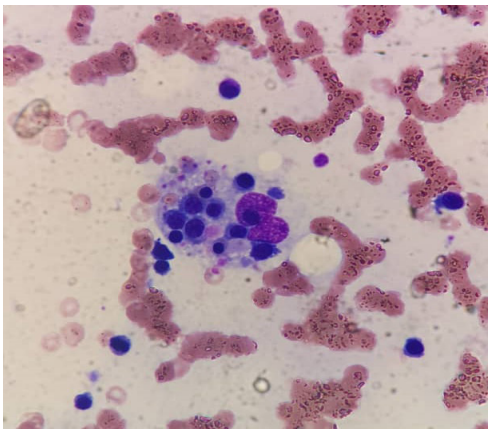

Figure 1: Bone marrow aspiration shown histiocyte with prominent haemophagocytic activity (May-Grünwald-Giemsa stain, magnification 400x).

On D9 of hospitalisation HLH was suspected as serial full blood count showed persistent thrombocytopenia with platelet count ranging from 22-54 x 10^9/L despite being given 12 units of platelet via transfusion. Haemoglobin level dropped from the initial level of 13.5 to 9.2 g/dL with no evidence of haemorrhage. Additional blood tests were sent to look for evidence of HLH. Full blood picture showed toxic granulation of neutrophils and reactive lymphocytes. Further blood investigations showed hypofibrinogenaemia (1.1 g/L; reference range 2.1-3.9 g/L), hypertriglyceridaemia (4.51 mmol/L; reference <1.7 mmol/L), hyperferritinaemia (10,382.26 µg/L; reference range 4.63-204 µg/L).

Bone marrow aspiration was done on D11 of hospitalisation. The result showed increased lymphocytosis and histiocytes, some with prominent haemophagocytic activity, ingesting numerous cells of all three haemopoietic lineages (Figure 1).

On D10 of hospitalisation patient was started on intravenous immunoglobulin (IVIG) 0.4 mg/kg/day for five days and started on IV methylprednisolone 50 mg daily for two days. The steroid treatment was changed to IV dexamethasone as haemoglobin level continue to drop to 6.4 g/dL. IV dexamethasone was given in tapering doses, 4 mg thrice daily for a week, then 4 mg twice daily for a week, then 4 mg once daily for a week, then 4 mg every other day for a week. Total of two units of packed red blood cells

was transfused, with the last transfusion on D27 of hospitalisation. After the treatment for HLH, her condition improved. She was extubated on D22 of hospitalisation and transferred to the general ward the following day. Her cytopenia improved. She was discharged on D47 of hospitalisation with WBC 15.2 x 10^9/L, haemoglobin 10.5 g/dL, and platelet 277 x 10^9/L. Her ferritin decreased to 2937.10 µg/L, while fibrinogen increased to 4.3 g/L. One month after discharge from hospital, during follow-up at the haematology clinic, her ferritin level further decreased to 1081.85 µg/L.

DISCUSSION

HLH or HPS is a rare but potentially life-threatening syndrome of excessive immune activation leading to cytokine storm. Due to nonspecific clinical presentation and nonspecific changes in laboratory results, diagnosis of HLH often delayed. HLH-2004 trial introduced the diagnostic criteria for HLH (Table 1) (Henter et al. 2007). Other than the molecular diagnosis of the HLH, none of the other investigation result is specific for HLH. Haemophagocytosisis is not pathognomonic for HLH, and might not appear in the early stage of the disease. Hyperferritinaemia can occur in other disorders, such as haemochromatosis, frequent blood transfusion and haematological malignancies. A scoring system known as HScore has been developed to calculate the probability of having HLH (Fardet et al. 2014).

The exact prevalence of HLH in dengue patients in Malaysia is not clear. However, a 5-year retrospective single-center study in all adult patients with severe dengue admitted to a tertiary ICU in Malaysia reported that 31 out of 180 patients (17%) with severe dengue fulfilled 4 or more criteria by HLH-2004 trial, while 21 out of 180 patients (12%) with severe dengue had HLH as defined by HScore (Kan et al. 2020).

Kan et al. (2020) suggested that HLH needs to be considered in patients with dengue and with persistent fever, cytopenia, severe organ involvement, drastic increase in liver enzymes, and/or altered mental state, in particular if ferritin is greater than 10,000 µg/L. McClain & Eckstein (2020) suggested a modified criteria to diagnose HLH: 3 out of 4 clinical findings (fever, splenomegaly, cytopenias, hepatitis) plus abnormality of 1 of 4 immune markers (haemophagocytosis, hyperferritinaemia >3,000 µg/L, hypofibrinogenaemia, absent or very decreased NK cell function).

In this case, HLH was suspected in view of persistent cytopenia with elevated liver enzyme and renal failure. She fulfilled 5 out of 8 criteria by HLH-2004 trial (fever, cytopenia, hypertriglyceridaemia or hypofibrinogenaemia, haemophagocytosis in bone marrow aspirate, hyperferritinaemia). Her HScore was 281, with the probability of having HLH being 99.9%. She also fulfilled the modified criteria by McClain & Eckstein (fever, cytopenia, hepatitis with abnormality of 3 immune markers)(McClain & Eckstein 2020).

When secondary HLH is diagnosed in a patient, Epstein-Barr virus (EBV)

infection and malignancy such as lymphoma, leukaemia should be excluded (Wang et al. 2017). EBV IgG for this patient was positive but her EBV IgM was negative with no detectable amount of EBV-specific DNA in her blood sample. Both peripheral blood film and bone marrow aspiration result of this patient were not suggestive of haematological malignancy. Detection of both dengue IgM and IgG in this patient suggested that she has a secondary dengue infection, with the HLH most likely being triggered by this.

Mortality-associated risk factors in dengue-associated HLH includes peak values of AST, ALT, lactate dehydrogenase (LDH), ferritin and creatinine, lowest platelet counts, hepatomegaly, severe organ involvement, severe bleed, severe leak, increasing age at hospitalisation, acute physiology, Age, Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II) and Sequential Organ Failure Assessment (SOFA) scores (Kan et al. 2020).

Treatment protocol for HLH which uses the combination of corticosteroid, chemotherapy agent and immunosuppressant such as HLH-94 and HLH-2004 is available but designed mainly for paediatric population (Henter et al. 2007; La Rosée 2015). There is no standardised treatment protocol available yet for HLH in patient with dengue. Kan et al. (2020) suggested a short course of HLH-directed therapy in view of high dengue-HLH mortality. Specific treatment for HLH involves corticosteroid, IVIG or the combination of both. Choices of corticosteroid is either prednisolone, methylprednisolone or dexamethasone. In this patient, judicious intravenous fluid therapy was given for severe dengue while broad spectrum antibiotic was empirically administered to cover possible superimposed bacterial infection. Intravenous dexamethasone and IVIG was given for this patient showing good response.

Other reported cases of dengue-associated HLH showed variable responses to supportive treatment and specific treatment. Two patients with milder form of dengue-associated HLH recovered with supportive treatment (Tan et al. 2012). Specific treatment with corticosteroid and/or IVIG showed clinical and biochemical improvement in patients with dengue with HLH and MOD (Wan Jamaludin et al. 2015; Ab-Rahman et al. 2015; Tan et al. 2012; Srichaikul et al. 2008; Sorakhunpipitkul et al. 2011). Kan et al. (2020) reported that 13 of 25 (52%) patients who had dengue-HLH and received corticosteroids survived, including 8 of 10 (80%) who were administered dexamethasone.

Etoposide, a chemotherapy agent, is used as part of the treatment protocol for HLH in HLH-94 and HLH-2004 (Henter et al. 2007). Treatment with both dexamethasone and etoposide showed substantially reduced mortality in severe EBV-associated HLH (Imashuku et al. 2003; Imashuku et al 2004; Kogawa et al. 2014). Kan et al. (2020) suggests the early use of steroid with possibe addition of etoposide in dengue-associated HLH patients with more pronounced hyperinflammation

or rapidly deteriorating status, in particularly in patients with severe liver involvement. In this patient, etoposide was not used in the treatment because patient responded well to IV dexamethasone and IVIG, with the treating team not being familiar with the use of etoposide in HLH patient.

CONCLUSION

HLH in adults with dengue infection is a rare but potentially life-threatening syndrome of excessive immune activation that might be overlooked because of the nonspecific clinical presentation. Early suspicion of HLH in patients with dengue infection is vital. Routine use of diagnostic criteria from the HLH-2004 trial and HScore can be used in dengue with MOD to confirm the diagnosis of dengue-associated HLH. Early treatment of HLH in dengue with corticosteroid and/or IVIG may improve clinical and biochemical outcome, but further studies are required to validate its use.

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