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Modeling of hemoglobin in dengue fever and dengue hemorrhagic fever using bioelectrical impedance

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Introduction

Dengue viruses are arthropod borne flaviviruses that cause significant morbidity and mortality in tropical and subtropical regions of the world. Dengue fever (DF) is a self-limited illness characterized by fever, headache, myalgia and arthralgia. Dengue hemorrhagic fever (DHF) is an infection associated with an increase in microvascular permeability, a decrease in plasma volume, and in severe forms hypotension and shock (Nimmannitya 1987, Halstead 1989, Bethell *et al* 2001, Ibrahim *et al* 2001).

Measurement of total hematocrit (Hct) (Hct increased to 20%) or hemoglobin (Hb) concentration (Hb above the upper normal range limit) is one of themethods formonitoring the onset and progression of plasma leakage in dengue patients (WHO1997). Thesemeasurements will determine the degree of microvascular permeability rate in the patients. At present, the clinical monitoring management in the dengue patients is to take the patient’s serum sample and determine and monitor the Hct or Hb value. This technique is invasive, requires precious personnel time for drawing blood samples and may introduce a substantial delay before data are available, depending on the proximity of the testing laboratory. Moreover, frequent blood taking will cause further injury to the subcutaneous tissue, therefore affect the capillary permeability and also contribute to dilutional anemia. Thus this technique is potentially risky for the DHF patient, especially when in a state of bleeding.

This paper introduces a novel approach to model hemoglobin in DF and DHF patients using bioimpedance analysis (BIA) technique. BIA is a safe, simple, inexpensive and noninvasive technique to estimate body composition and body hydration in human (Ibrahim *et al* 2002, Thomasset 1962, Lukaski *et al* 1985, Nigrelli and Pizzarelli 1989, Klassen *et al* 2000). The purpose of this study was to investigate and determine whether BIA measurement is more reliable or has any significant predictor to estimate changes in Hb volume, compared to current conventional clinical practice. Since Hct and Hb (Hct is approximately 3Hb (Behrman *et al* 2002, Nelson *et al* 1969)) are related to each other, and a recent study by Ibrahim *et al* (2003a) has shown that Hct was not promising for modeling DF and DHF patients, thus Hb was chosen for the modeling for its accuracy status where the latter requires the baseline value in order to monitor the increment percentage (WHO 1997).

Arecent study conducted by Libraty *et al* (2002) has shown that usingwater compartments aspects in BIA did not improve on serial hematocrit and bodyweight determinations for monitoring plasma volume contraction and extracellular expansion, respectively. However, studies conducted by Klassen *et al* (2000), Mazariegos *et al* (2000) and Ibrahim *et al* (2002) have shown that BIA was sensitive in determining the hydrational profile in dengue patients. Klassen *et al* (2000) and Mazariegos *et al* (2000) findings using Cole–Cole plots of reactance versus resistance indicated that the multi-frequency BIA was not only for defining hydrations profile in a systemic disease such as dengue but also for serial monitoring for dengue patients in acute and recovery phase.

In this study, we focused on various combinations of the whole body impedance measurements (reactance (X_c), resistance (R), body capacitance (BC), phase angle (α)), mass distribution and water compartments, the patient's demographic data and symptoms perspectives to be the independent variables to predict Hb. This paper presents the development of Hb predictive equation on the day fever subsided in patients with DF and DHF using single frequency of BIA.

Theory

Blood is a living tissue that has α, β and γ frequency dispersions. The β dispersion is known as a structural relaxation and occurs at the radio-frequency range of 10 kHz to 10MHz (Pethig 1987, Schwan 1957). At 50 kHz, a proportion of applied current is unable to penetrate the cell membranes, therefore passes only through the extra-cellular space. The impedance of blood is almost constant at frequencies below 100 kHz, since the electric current passes through the plasma only, while the membranes of blood cells act as insulators.

Many previous studies on the electrical impedance of blood were carried out at only one frequency below 100 kHz. Since blood cells do not conduct at these low frequencies, the measured resistivity related only to the properties of plasma and the volume concentration of blood cells. The method was used to measure hematocrit (Okada and Schwan 1960, Ibrahim *et al* 2003a), cardiac output (Kubick *et al* 1960), erythrocyte sedimentation rate (ESR) (Nelson and Wilkinson 1972), etc. It was extended to monitor fluid volume (Tender 1985) and to measure other body tissues.

In healthy subjects, since at a low frequency of 50 kHz the membranes of blood cells will act as insulators, the Hb is unable to be measured; however in DHF patients, due to the pathophysiology of the dengue diseases that lead to a plasma leakage, low thrombocytopenia and coagulopathy (WHO 1997), it is possible to estimate the Hb volume indirectly using the BIA technique.

The BIA technique is based on the passing of a low-amplitude electrical current less than 1 mA (500 to 800 μA) with frequency (50 kHz) and it measures the whole body's resistance (R), reactance (X_C), impedance (Z) and phase angle (Kushner 1992, Bourne 1996). Rigaud *et al* (1996) obtained several results for BIA whole body measurements in the frequency range 1 kHz–10 MHz. They showed that the BIA can measure the muscle impedance of the whole body at frequencies below 100 kHz. This technique of passing an electrical current through the body allows the perception of membrane disturbances (Lukaski 1987, Kushner 1992). The reactance in the BIA reflects the dynamic performance of the cellular membrane structure (Bottoni *et al* 2003, Leidtke 1998, Mattar 1996). The smaller the semi-permeable membrane volume or smaller the quantity of membranes the greater the reactance. Generally, high reactance values from BIA measurement indicate better health and cell membrane integrity. A recent study conducted by Ibrahim *et al* (2003a) indicates that reactance had a significant direct effect on the evaluation of hematocrit status in DHF patients while the experimental findings conducted by Ibrahim *et al* (2003b) also indicate that reactance is the key determinant indicator for classifying the risk category in the DHF patients. Thus, reactance has been used as a good indicator for assessing the condition of the patient's health (Nyboer *et al* 1940, Nyboer 1970, Matter 1996, Ibrahim *et al* 2003b, Bottoni *et al* 2003, Magariegos *et al* 2000, Klassen *et al* 2000).

In DHF patients, an increased vascular permeability that gives rise to loss of plasma from the vascular compartment into the extravasular tissue, will result in haemoconcentration (i.e. an increased concentration of Hb) in the blood vessel (WHO 1997). These significant changes of vascular permeability lead to the alteration function in the cellular membrane in maintaining the fluid osmotic pressure and ion concentration gradient between the intracellular and extracellular compartments (Bottoni *et al* 2003, Ganong 2003, Leidtke 1998). This mechanism affects the reactance and indirectly reflects an increased concentration of Hb.

Patients and methods

3.1. Patients

Eighty-three adult patients aged 12 years and above, with serological confirmation of acute dengue infection, admitted to HUKM during the years 2001 and 2002, were prospectively studied. All patients were asked to abstain from eating and drinking for 4 h prior to the BIA measurement.

3.2. Methods

Informed consent was obtained from each patient and anthropometrics measurements (height and weight)were taken at admission; however, theweightwasmeasured daily until discharged. Clinical and epidemiological data were recorded by the investigator using standardized questionnaire data collection forms designed for the study (Ibrahim *et al* 2001). The clinical data comprise of data onset of fever, symptoms, daily surveillance of symptoms, signs, physical examination, blood results from laboratory tests and the bioelectrical impedance measurements.

Patient serum samples were tested for hemoglobin determination using an automated counter (Coulter STKSmachine) and also tested for serological evidence of acute dengue virus infection by detection of IgM (primary infection) and IgG (secondary infection) (WHO 1997) antibody using an enzyme-linked immunosorbent assay (ELISA)method (Chungue *et al* 1989) or using the PanBio dengue Duo IgM and IgG rapid strip test (PanBio 2001). Hemagglutininhibition (HAI) assays were performed in the University of Malaya Medical Center, Kuala Lumpur when the finding of the ELISA method was not conclusive (Lam *et al* 1987, WHO 1997).

3.2.1. BIA measurement.

Patients were asked to lie supine on their bed and two electrodes were placed on the patient's right hand, one at the base of the knuckles and another slightly above the wrist joint. Another two electrodes were placed on the right foot, one near the base of the toes and the other slightly above the ankle joint. A constant current less than 1 mA at a single frequency of 50 kHz (biodynamics) was injected to the base of the knuckles and base of the toes and the signal was picked up by the other two sensor electrodes (slightly above the ankle and wrist joint).

Each measurement took approximately 3 min. Bioelectrical impedance measurement was performed with a biodynamic Model 450 bioimpedance analyzer, from Biodynamic

Corporation, USA. The patient profile such as age, sex, height and weight was entered into the BIA 450 analyzer. The analyzer directly measures the resistance (*R*), reactance (X_c), body capacitance (BC) and phase angle and uses regression analysis to compute the mass distribution and water compartments.

3.2.2. Study protocol definition

. Since the patients were admitted at different stages of their illness, their daily progress was dated with reference to the day of defervescence. Hence, fever day 0 is defined as the day of fever subsidence when the body temperature fell below 37.5 ◦C. Days prior to fever day 0 were designated as fever day −1 (1 day before fever subsided), fever day −2, etc. Days after the fever subsided were designated as fever day +1 (1 day after fever subsided), fever day +2 and onwards (Ibrahim *et al* 2003b).

The model for Hb in dengue patients was evaluated on fever day 0 because during and shortly after the fall in temperature, most patients become very ill and the condition of patients who progress to shock will suddenly deteriorate rapidly (WHO 1997).

Statistical analysis

The statistical analysis was performed using SPSS statistical package version 10.01 for Windows 1998. Simple linear regression was used in the preliminary analysis for testing the significance of the variables. These variables were then included in the multivariate analysis. Multiple linear regression was used to analyze the control effects of the patient demographic and symptom variables and BIA parameters on Hb. The model was constructed in three steps as follows:

(a) When correlation exists between variables, one or more variables were excluded for the multivariate analysis.

(b) The demographic variables were first included in the model. Once the demographic predictors were identified, the BIA parameters were added in and which of these parameters were important predictors was determined.

(c) The last step was to include symptoms and find out whether with the addition of this predictor will make further significant contributions or not.

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