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Evaluation of the URIT-2900 Automated Hematology Analyzer for screening of thalassemia and hemoglobinopathies in Southeast Asian populations

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ABSTRACT

Background: The effectiveness of the URIT-2900 Hematology Analyzer for screening of hemoglobinopathies commonly found in Southeast Asian populations was examined.

Methods: Appropriate cut-off values of MCV and MCH for screening of α^0 and β thalassemias were derived from the receiver operator characteristic curve conducted initially on 279 subjects with various thalassemia genotypes. Validation was performed additionally in a cohort of another unrelated 313 subjects.

Results: The best cut off values of MCV and MCH were found to be 78 fL and 27 pg, respectively. Using these cut off values in combination with the dichlorophenolindophenol test in screening of α^0 thalassemia, β thalassemia and Hb E in a cohort study revealed 100% sensitivity, 79.6% specificity, 80.0% positive predictive value and 100% negative predictive value.

Conclusion: The combined blood cell counting using the URIT-2900 Automated Hematology Analyzer and dichlorophenolindophenol test is suitable for population screening of thalassemia and hemoglobinopathies in Southeast Asia.

Introduction

Thalassemia and hemoglobinopathies are the most common inherited disorders among humans and represent a major public health problem in many areas of the world including Southeast Asia. In these areas, the three thalassemia diseases including homozygous α^0 thalassemia (Hemoglobin (Hb) Bart's hydrops fetalis), homozygous β thalassemia and β thalassemia/Hb E are the prime targets of the prevention and control [1,2]. The aim of screening is therefore to identify carriers with α^0 thalassemia, β thalassemia and Hb E. As a general guideline, primary screening of α^0 thalassemia and β thalassemia involves accurate blood cell counting using standard electronic blood cell counter [3] whereas Hb E can be identified by the dichlorophenolindophenol (DCIP) dye test [4,5]. Positive samples need further confirmatory test while negative samples can be eliminated from further complicated and expensive testing. Alternatively, this could be done using a combined osmotic fragility (OF) test and DCIP test. This, however, could be problematic in the areas. While OF test is highly observer dependent and difficult to standardize, the expense of accurate blood cell counting usually precludes the possibility of expensive electronic blood cell counters.

The URIT-2900 Automated Hematology Analyzer is a relatively cheap multi-parameter hematology analyzer designed and developed in China. In Thailand, the cost of performing a complete blood count (CBC) with this machine is approximately 1 US dollar as compared to 3–5 US dollars with other standard analyzers. The URIT-2900 Automated Hematology Analyzer can conduct continuous measurements on pre-diluted or whole blood specimen in batch, forwarding the measurement data in 19 parameters including total numbers of white blood cell (WBC), lymphocyte (LY#), monocyte (MO#) and granulocyte (GR#) and percentages of lymphocyte (LY%), monocyte (MO%) and granulocyte (GR%) as well as red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-repeat precision (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT). Data could be analyzed, arranged automatically, forming histograms on white blood cell of thee categories, RBC and platelet. The parameters of blood test are expressed in three ways including direct way, histogram and derivation from certain formula. MCV is expressed in direct way and MCH is derived from the formula; Hb/Rbc. We evaluate this analyzer in screening of thalassemia and hemoglobinopathies in Thailand.

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Materials and methods

Subjects

Study subjects included initially 279 routine blood specimens at our thalassemia diagnostic laboratory at Khon Kaen University for use to establish appropriate cut off values. Additional 313 blood specimens used in validation study were obtained from high school students from another project on the study of anemia in northeast Thailand [6]. All blood specimens were collected using EDTA as anticoagulant. Blood specimens with different MCV and MCH values of known thalassemia genotypes in our series were also included for study on the effect of aged specimen. Ethical approval of the study protocol was obtained from the Institution Review Board of Khon Kaen University, Thailand (HE 522238).

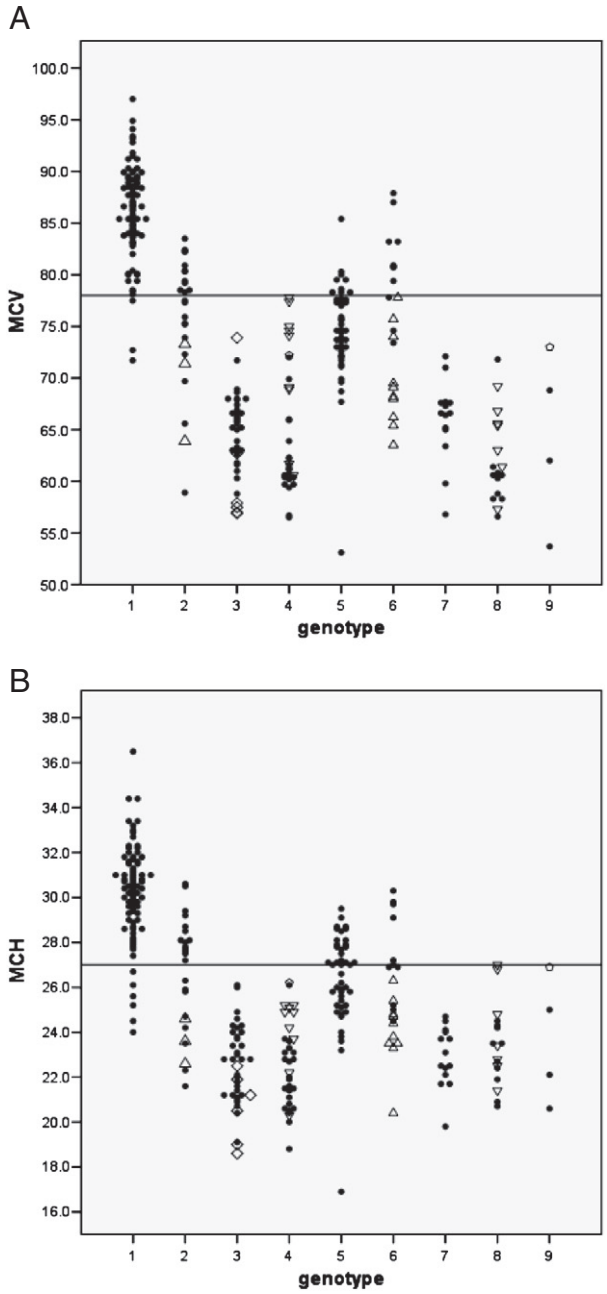

Fig. 1. Scatter plots of MCV (A) and MCH (B) values of subjects with known thalassemia genotypes for ROC curve construction. 1: non thalassemia (n = 82), 2: α^+ thalassemia [●: heterozygous α^+ thalassemia (n = 22), △: homozygous α^+ thalassemia (n = 3)], 3: α^0 thalassemia [●: heterozygous α^0 thalassemia (n = 33), : Hb H disease (n = 6)], 4: β thalassemia [●: β thalassemia trait (n = 23), ▽: β thalassemia trait with α thalassemia (n = 8), O: δβ thalassemia trait (n = 1)], 5: Hb E trait (n = 45), 6: Hb E trait with α^+ thalassemia [●: heterozygous α^+ thalassemia (n = 11), △: homozygous α^+ thalassemia (n = 10)], 7: Hb E trait with α^0 thalassemia (n = 14), 8: Homozygous Hb E [●: Hb EE (n = 10), ▽: Hb EE with α^+ thalassemia (n = 7)], 9: β thalassemia/Hb E [●: β thalassemia/Hb E (n = 3), O: δβ thalassemia/Hb E (n = 1)].

Laboratory investigation

Erythrocyte indices were collected on the URIT-2900 Automated Hematology Analyzer (URIT Medical Electronic (Group) Co., Ltd., Guilin, Guangxi, P.R. China) within 2 h after blood collection. For aging study, specimens were stored at room temperature or refrigerated at 4 °C for 0–7 days before analysis. DCIP test for Hb E was performed in all samples as described in [4,5]. Hb analysis was done using an automated capillary electrophoresis (Capillarys 2, Sebia, France) [7] or column chromatography (Drew Scientific co., Ltd, UK). Plasma ferritin levels were obtained using the Syncron LXi® 725 Access® Clinical system (Beckman Coulter, United States of America). Plasma ferritin of less than 20 μg/L was considered as iron deficiency [8]. Identification of the α^0-thalassemia (SEA and THAI deletions), α^+-thalassemia (3.7 and 4.2 kb deletions), Hb Constant Spring and Hb Paksé was routinely performed in our laboratory using PCR methods described elsewhere [9,10]. Common β thalassemia mutations in Thailand were also investigated in samples with elevated Hb A_2 by using allele specific PCR [11].

Statistical analysis

To determine suitable cut-off values of MCV and MCH for thalassemia screening, the receiver operator characteristic (ROC) curve was plotted using MedCalc software version 11.2.1.0 (MedCalc Software, Inc., Mariakerke, Belgium). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated in order to assess the effectiveness of thalassemia screening using the results of Hb and DNA analyses as gold standards.

Results

Fig. 1 demonstrates the distribution plots of MCV and MCH values of the initial 279 subjects with various globin genotypes. As many as 41 genotypes were included. As the prime targets for screening in the region are α^0-thalassemia, β-thalassemia and Hb E, subjects were grouped and values were plotted according to these three important alleles as described in the legend of the figure. As expected both MCV

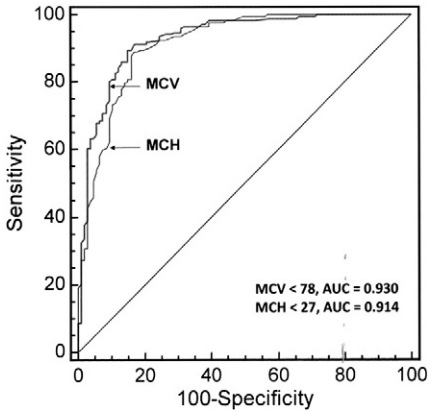

Fig. 2. The ROC curve of MCV and MCH for thalassemia screening using the URIT-2900 Hematology Analyzer.