Application of an Automated Hemoglobin Analyzer, HLC-723G7, for Clinical Samples

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Accepted for publication on January 25, 2005

ABSTRACT. An automated hemoglobin analyzer, HLC-723G7, was introduced to determine Hb A_2 and Hb F in a total of 276 clinical blood samples from 60 antenatal care attendants (pregnant mothers), 45 children with dengue hemorrhagic fever or acute viral infection, 41 old fild survey samples, 32 blood donors, 29 cases who had reduced osmotic fragility and/or reduced MCV, 22 stroke patients/controls, 17 known thalassemia patients, 13 apparently healthy volunteers, 12 cardiovascular disease patients/controls, and 5 known diabetics. The HLC-723G7 has many advantages over conventional methods (cellulose acetate electrophoresis and elution) for the determination of Hb A_2. Some information, such as hemoglobin patterns (normal and/or abnormal) and their relative contents, is also obtainable in addition to Hb A_2 and Hb F contents. Furthermore, Hb A_2 contents, are also readily provided in Hb E traits (Hb E heterozygotes), which is not possible with the conventional methods. Based on the findings from this initial and introductory work with the HLC-723G7, it can be concluded that this machine should be of great value and highly beneficial in future thalassemia and hemoglobinopathy research in Myanmar.

Key words : automated hemoglobin analyzer, HLC-723G7 — Hb A_2 value — α-thalassemia — β-thalassemia — abnormal hemoglobin

Thalassemia and hemoglobinopathies are highly prevalent in Myanmar as in other Southeast Asian countries.[1] Diagnosis of thalassemia major and severe thalassemia intermedia is not problematic, establishing a diagnosis of thalassemia traits is difficult because of its asymptomatic nature and near normal or normal hematological indices. Increased Hb A_2 ($>4.0\%$) is a β-thalassemia trait that is used in diagnosis. Reduced Hb A_2 content ($<2.5\%$) is often found in α-thalassemia and iron deficiency anemia.[2-5]

An automated hemoglobin analyzer, HLC-723G7 (Tosoh Co., Tokyo, Japan) was provided to the Pathology Research Division, Department of Medical Research (Lower Myanmar) to encourage to the running of thalassemia and hemoglobinopathy projects in Myanmar. Since its

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installation and successful set-up, in the initial and preparatory phase of major projects, the machine has been used with clinical blood samples for the determination of Hb A_2. The results and findings are described and discussed in detail below.

MATERIALS AND METHODS

Following to the manufacturer's instruction,[6] Hb A_2 was determined in a total of 276 blood samples from 60 pregnant mothers, 45 children with dengue hemorrhagic fever (DHF) and acute viral infection (AVI), 41 old field survey whole blood and cord blood samples, 32 blood donors, 29 children or adults with hemolytic features, 22 strokes patients and controls, 17 known Hb H disease cases, 13 volunteers, 12 cardiovascular disease (CVD) patients and controls, and 5 known diabetics. Field survey samples and some of the DHF/AVI samples were old hemolysates.

RESULTS AND FINDINGS

The machine printed out chromatograms with the following findings:

(a) The retention time of Hb A_2 in normal controls was about four minutes, while that of Hb F was about one minute (Fig 1).

(b) Hb A_2 could be detected in all samples with a value ranging from 0.1% to 7.9% of the total Hb. The cases having a lower Hb A_2 value of less than about 2.0% were frequently diagnosed as α-thalassemia (Fig 2A) and those having one of more than 4.0% were diagnosed as β-thalassemia (Fig 2B).

(c) The Hb E heterozygote (Hb EA) was observed in 53 samples where Hb

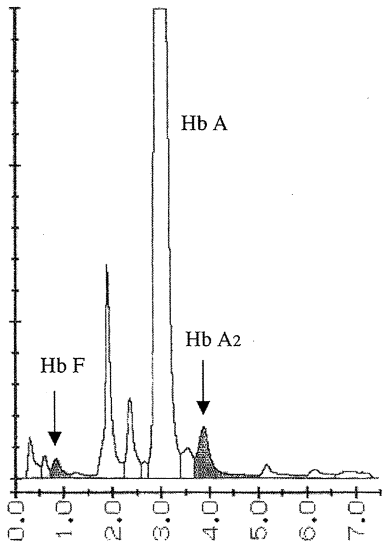

Fig 1. A chromatogram of a control sample obtained by the HLC-723G7. The retention times of Hb F, Hb A and Hb A_2 were around 1.0, 3.2 and 4.0 minutes, respectively. In this case, the values of Hb F and Hb A_2 were respectively estimated to be 1.0% and 3.3% of the total Hb.

A_2 could also be estimated ranging from 1.6% to 3.9% (Fig 3). This machine could separate Hb A_2 from Hb E but was not capable of determining an accurate Hb A_2 value.

(d) The Hb E homozygote (Hb EE) was seen in five samples where the Hb A_2 peak was not only seen but where the Hb A peak also disappeared (refer to Fig 6). The Hb A_2 peak might be included in the large Hb E peak.

(e) Abnormal looking peaks having a shorter retention time than that of the Hb A peak were observed in 24 samples. One of them is shown in

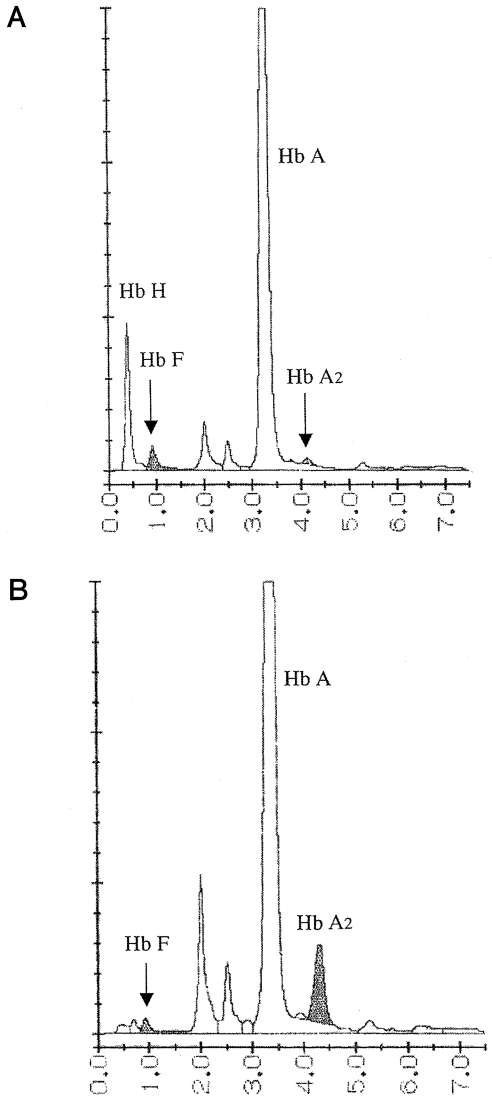

Fig 2. A : This case had a lower Hb A_2 value (0.8%) and a faster eluted Hb (8.5%) which is considered to be the Hb H peak. B : This case had a higher Hb A_2 value and was diagnosed as β-thalassemia. The Hb F and Hb A_2 values estimated from the chromatogram peaks were 1.3% and 6.0%, respectively.

Fig 4. These Hbs should be further studied in detail.

(f) Abnormal looking peaks having a longer retention time than that of the Hb A peak were observed in 29 samples. One of those exhibited behavior like that of Hb C (Fig 5). These Hbs should be further studied in detail.

(g) Hb C and Hb D peaks were seen in all of the old hemolysate samples

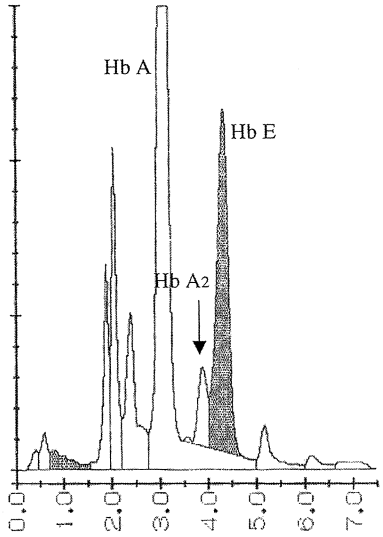

Fig 3. A chromatogram obtained from a patient with an Hb E heterozygote (Hb AE). The Hb A_2 peak appeared ahead of the Hb E peak. From this chromatogram, the Hb A_2 value was estimated to be 3.9%, while the respective values of Hb F and Hb E were 1.5% and 23.4% of total Hb.

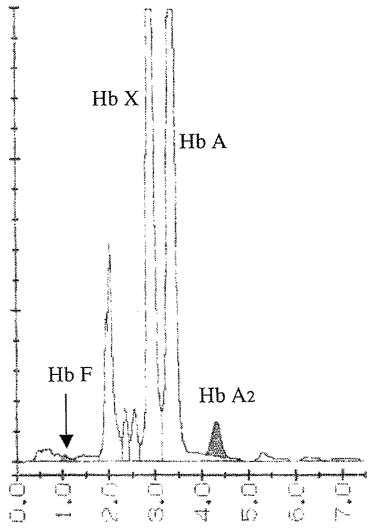

Fig 4. This case had a faster abnormal looking Hb peak (Hb X) ahead of the Hb A peak. The content was about 40% of the total Hb, while the Hb F and Hb A_2 values were 0.4% and 2.7%, respectively.

of field surveys and six samples of DHF/AVI children. Some unknown slower and faster peaks were also noted in these samples (Fig 5).

(h) Hb F was detected in all of the samples ranging from 0.1-44%. Very high peaks were observed in the cord and placental blood samples, in which high peaks of Hb H or Hb Bart's appeared (Fig 6).

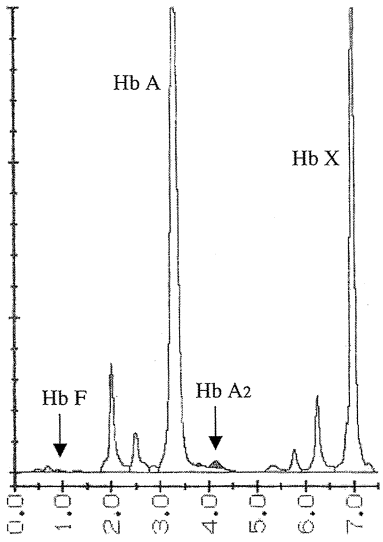

Fig 5. This case had an abnormal looking Hb peak with a long retention time like Hb C (Hb X). The content was about 32% of the total Hb, while the Hb F and Hb A_2 values were near 0% and 0.8%, respectively.

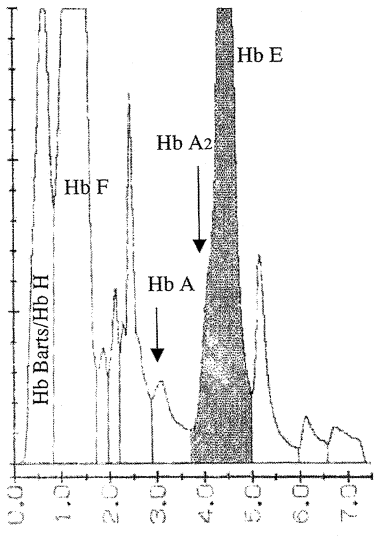

Fig 6. A chromatogram obtained from a placental blood sample of a pregnant mother with a Hb E homozygote (Hb EE). The Hb A peak had disappeared on the figure. The Hb A_2 peak was included in the Hb E peak, which was estimated to be 39%. The large peaks appearing around 0.5 and 1.0 minutes were considered to be Hb Bart's or Hb H (17%) and Hb F (44.2%), respectively.

DISCUSSION

The HLC-723G7 has provided quite a lot of information on both normal and abnormal hemoglobin pattern to β-thalassemia. Its determination of Hb A_2 content is accurate and precise. Reliability and reproducibility is very high. The advantages to this machine over the conventional methods used for Hb A_2 determination are numerous, including the delivery of fully automated sample and results printed out within eight minutes (Fig 1). The per sample cost is acceptable and reasonably cheap. From the technical point of view, neither special skill nor training is required to handle the machine. Sample preparation is also very easy and hemolysates can be prepared by the washing reagent provided or distilled water.[6] Whole blood using any anticoagulant is acceptable.

The HLC-723G7 readily determines Hb A_2 content separately even in Hb E heterozygotes (Fig 3). This is extremely useful because the conventional elution methods reveal Hb A_2 content around 10%, which is the margin to differentiate Hb A_2 and Hb E. This can result in diagnostic confusion and dilemma. Now this problem can be easily solved by determination with this machine. The determination of the prevalence rate for Hb E and/or β-thalassemia traits should become more accurate with the use of this machine in prevalent studies (Fig 2B, 3 and 6).

The abnormal looking peaks found in this initial work need to be characterized further by DNA and/or peptide analysis (Fig 4 and 5). It was noted that all of the old field survey hemolysate samples were prepared using carbon tetrachloride (CCl_4) and showed similar abnormal hemoglobin peak patterns. It would be interesting to determine whether CCl_4 has any effect on globin chain structure or conformation on a long-term basis.

Thalassemia and hemoglobinopathies of different forms are highly prevalent in Myanmar, they vary among different indigenous groups and are also heterogeneous at the clinical, hematological and molecular levels.[7-10] We intend to continue this initial and introductory work in Myanmar with future collaboration planned in research involving detailed prevalence and epidemiological studies at the clinical, hematological and molecular levels using the HLC-723G7. The machine has been found to have many advantages, and it is cost-effective for obtaining diagnostic evidence of thalassemia and hemoglobinopathies in any clinical setting.

ACKNOWLEDGMENT

We wish to thank Mr. Alvan Chan, Sysmex Singapore PTE Ltd., for his kind technical assistance in the installation of the HLC-723G7, and Miss. Khin Myo Sett and Mrs. Myat Mon Oo for determination of the Hb A_2 value by the cellulose acetate electrophoresis-elution method.

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