USING RETICULOCYTE INDICES TO IDENTIFY α-THALASSEMIA– A PRELIMINARY REPORT

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Abstract. Thalassemia poses an important public health problem in Thailand. The Ministry of Public Health of Thailand has conducted many programs for the control of thalassemia. These programs are useless if there is no effective, affordable method of screening for carriers. We report on the possibility of using an automated reticulocyte analyzer in order to identify α-thalassemia. Thirty-one control subjects and 14 couples, whose offsprings were affected with thalassemia H (7 α-thalassemia-1 and 7 α-thalassemia -2) were studied. We found that almost all reticulocyte parameters could be used as dianostic marker of α-thalassemia-1 carriers. Unfortunately, α-thalassemia-2 had only 5 parameters that were significantly different when compared to normal controls. This is the report to focus attention on the use of reticulocyte parameters in the screening of α-thalassemia.

INTRODUCTION

Thalassemia, a hemoglobin disorder, is the most common inherited disorder in Thailand (Flatz *et al*, 1965). At present, 500,000 Thai people suffer from this disorder, which is a major health problem in Thailand (Fucharoen and Winichagoon, 1997). This problem affects not only public health but also the economy of the country. If no specific control method are applied, then widespread disease can be expected. Carrier detection, genetic counseling, and prenatal diagnosis should be encouraged (WHO Working Group, 1983). The Ministry of Public Health, Thailand has established many programs for the control of thalassemia. However, these programs will be useless if there is no effective and affordable method of screening for carriers.

The two common types of thalassemia are α-thalassemia and β-thalassemia. A number of methods, including hemoglobin electrophoresis, are used to screen for these diseases (Kaewboworn, 1985; Paritpokee *et al*, 1999). However, α-thalassemias cannot be detected by simple hemoglobin electrophoresis; the gold standard method of screening for α-thalassemia is genotype study.

Some recent studies have provided information about the difference between mRNA ratios in the reticulocytes of normal adults and individuals with α-globin gene deficiencies. Some studies have mentioned the value of the reticulocyte count in the identification of hemoglobin disorders. (Onofrio, 1996; Paterakis, 1996; Smetanina *et al*, 1996; Peebies *et al*, 1987). We report on the possibility of using an automated reticulocyte analyzer to identify α-thalassemia.

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MATERIALS AND METHODS

Study population

We tested the possibility of using reticulocyte parameters in 14 couples whose offsprings were affected with thalassemia H (7 α-thalassemia-1 and 7 α-thalassemia -2). Twenty-one control subjects, who had normal blood cell indices, hemoglobin electrophoresis patterns and inclusion body studies, were recruited. All subjects gave the informed consent prior to the commencement of the study.

Measurement of reticulocyte parameters

An automated reticulocyte count analyzer was used: the automated hematology analyzer, Technicon-H3 RTX. The Technicon-H3 RTX is an automated blood cell analyzer that performs CBCs and reticulocyte counts using an optical method that is based on the measurement of scatter and absorption of helium-neon laser light. The automated reticulocyte count method requires a preliminary manual mixing of 3µl of blood with 3 ml of reticulocyte reagent, containing a surfactant, which sphere red blood cells and reticulocytes, and the nucleic acid-binding dye, oxazine 750, which selectively stains reticulocytes. After a 15-minute incubation, the prepared sample is aspirated through the automated analyzer flow cell, where three directors measure laser scatter, and absorption. On a two-dimensional cytogram of absorption versus low angle scatter, the stained reticulocytes are separated from unstained erythrocytes, platelets, and leukocytes by appropriate thresholds.

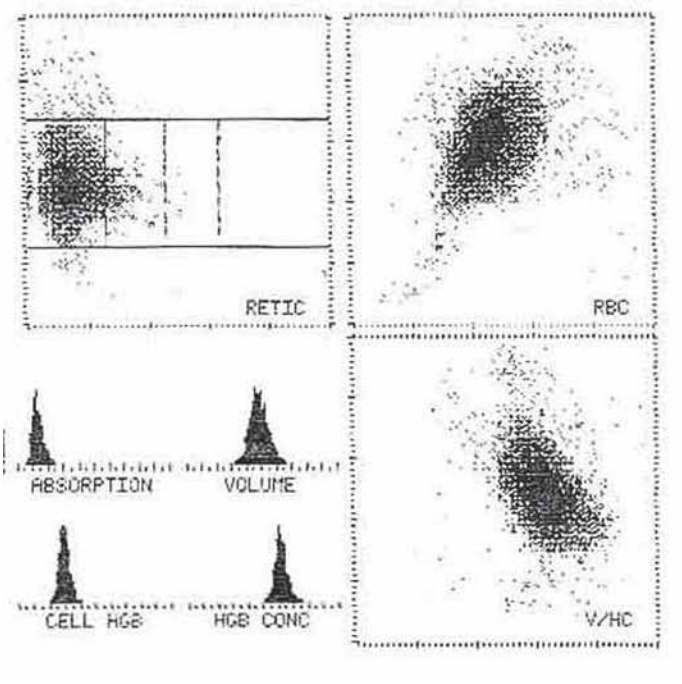

Fig 1- Graphic printout from a Technicon H 3 reticulocyte analyzer provides information regarding the staining intensity and cellular indices the of the reticulocyte population of the normal control group.

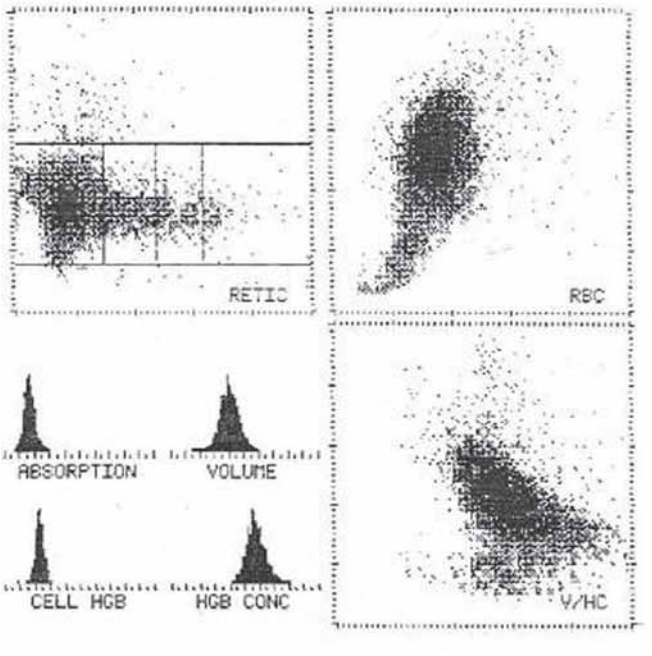

Fig 2- Graphic printout from a Technicon H 3 reticulocyte analyzer provides information regarding the staining intensity and cellular indices of the reticulocyte population of the α-thalassemia-1 group.

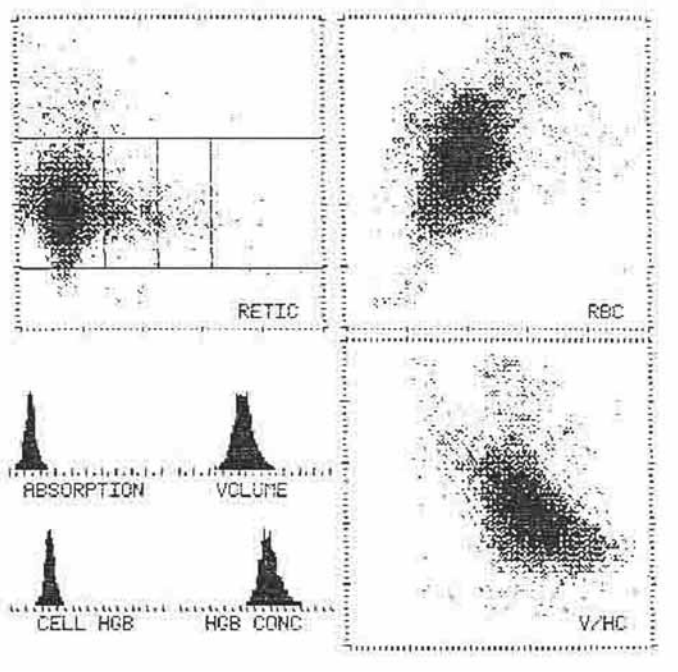

Fig 3- Graphic printout from a Technicon H 3 reticulocyte analyzer provides information regarding the staining intensity and cellular indices of the reticulocyte population of the α-thalassemia-2 group.

Table 1
Comparison of reticulocyte parameters between the α-thalassemia-1 group and the control group.

Parameters		Normal Mean ± SD (n=31)	α-Thal 1 mean ± SD (n=7)	p-value
Retic	(%)	1.36 ± 0.36	2.47 ± 1.24	0.009
L retic	(%)	83.58 ± 14.94	73.65 ± 9.06	0.000
M retic	(%)	11.61 ± 3.84	18.11 ± 5.04	0.007
H retic	(%)	1.87 ± 0.96	2.04 ± 3.10	0.002
MCVr	(fl)	113.95 ± 6.63	95.48 ± 4.68	0.000
CHCMr	g/dl	27.27 ± 1.38	24.9 ± 2.73	0.002
RDWr	(%)	17.13 ± 2.79	20.83 ± 3.99	0.005
HDWr	(g/dl)	3.22 ± 0.42	3.78 ± 0.44	0.003
CHr	(pg)	30.19 ± 2.50	22.87 ± 2.03	0.000
CHDWr	(pg)	4.1 ± 0.71	3.98 ± 0.95	0.697

Statistical significance: $p < 0.05$

Statistical analysis

All data were collected and analyzed. Descriptive statistical analysis was used when appropriate. Comparisons of the parameter avarages of both groups were made by unpaired *t* test; statistical significance was assumed at a p of 0.05.

RESULTS

Graphic printouts from a Technicon H3 reticulocyte analyzer showing staining intensity and cellular indices of the reticulocytes population in the normal control group, α-thalassemia-1 group, and α-

Table 2
Comparison of reticulocyte parameters between the α-thalassemia-2 group and the control group.

Parameters		Normal mean ± SD (n=31)	α-Thal 2 mean ± SD (n=7)	p-value
Retic	(%)	1.36 ± 0.36	1.87 ± 3.9	0.071
L retic	(%)	83.58 ± 14.94	79.51 ± 4.9	0.023
M retic	(%)	11.61 ± 3.84	15.00 ± 3.90	0.038
H retic	(%)	1.87 ± 0.96	3.88 ± 0.88	0.005
MCVr	(fl)	113.95 ± 6.63	101.77 ± 5.61	0.001
CHCMr	g/dl	27.27 ± 1.38	25.51 ± 1.16	0.315
RDWr	(%)	17.13 ± 2.79	19.06 ± 3.55	0.404
HDWr	(g/dl)	3.22 ± 0.42	3.62 ± 0.53	0.121
MCHr	(pg)	30.19 ± 2.50	25.10 ± 2.56	0.000
CHDWr	(pg)	4.1 ± 0.71	3.86 ± 0.65	0.358

Statistical significance: $p<0.05$.

thalassemia-2 group are shown in Figs 1, 2, and 3 respectively.

Almost all parameters could be used to identify a-thalassemia-1 carriers ($p<0.05$ in comparison to the normal control group) (Table 1). Unfortunately, a-thalassemia-2 carriers have only 5 parameters that are significantly difference to those of the normal controls (Table 2).

DISCUSSION

Thalassemia and hemoglobinopathies are transmitted by autosomal recessive genes (Flatz, 1965; Wasi *et al*, 1967, 1980; Fucharoen and Winichagoon, 1997). Research conducted in Thailand found that the prevalence of thalassemia genes is different in different parts of the country: α- thalassemia-1 trait is found in Bangkok at around 35% and in Chiang Mai at 12%. However, the α-thalassemia-2 trait in Bangkok is only 16%, while in Chiang Mai is 19%. β-thalassemia is found in Bangkok (3%), and in Chiang Mai (9%) and the northeastern region (2-3%). The prevalence varies by area because those who carry thalassemia genes may move around the country as migrants, married couples, and produce offsprings, many of whom will be carriers themselves. This phenomenon occurrs repeatedly.

Recently, Technicon has used flow cytometry, combined with the fluorescent staining, to categorize reticulocytes as L retic, M retic, and H retic, and to measure reticulocyte indices, such as MCV, CHCM, RDW, HDW, CH and CHDW respectively (Brugnara *et al*, 1994). We have tested the possibility of using these parameters to identify couples whose offsprings are affected with thalassemia H (genotype: α-thalassemia-1/ α-thalassemia-2).

This is the first data that supports the use of the technicon-H3RTX analyzer for this application. We recommended further research of these parameters for use in the diagnosis of α-thalassemia. Larger numbers of patients and other forms of thalassemia, iron deficiency, and anemia secondary to chronic diseases, ought to be evaluated.

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