RBC Count and Its Differentiation Potential among α-Thalassemia (SEA type), β-Thalassemia and HbE Heterozygotes

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ABSTRACT

In order to determine the differentiation potential of red blood cell parameters between severe and mild form of thalassemia heterozygotes, we have carried out automated blood cell analysis, one-tube osmotic fragility test (OFT), Hb H inclusion body test, hemoglobin identification by high-performance liquid chromatography (HPLC) and α-thalassemia 1 (Southeast Asian [SEA] type) genotyping in 58 thalassemia heterozygotes. Red blood cell (RBC) parameters in different thalassemia heterozygotes were compared. No difference in red blood cell (RBC) count was observed between α-thalassemia 1 (SEA type) and β-thalassemia heterozygotes. RBC count was significantly higher in α-thalassemia 1 heterozygotes (SEA type) and β-thalassemia heterozygotes than that in HbE heterozygotes. We concluded that the RBC count could not differentiate α-thalassemia 1 heterozygote (SEA type) from β-thalassemia heterozygote. However, if considered with MCV, MCH, MCHC and RDW, it provided great values in screening severe α-thalassemia 1 (SEA type) and β-thalassemia heterozygotes out of HbE heterozygote.

Key words : Red blood cell parameters, Red blood cell indices, Thalassemia screen, Thalassemia heterozygote, HbE heterozygote

INTRODUCTION

Thalassemia is a syndrome characterised by reduction or absence of globin chain synthesis, comprising two common types: α- and β-thalassemia. α-thalassemia is generally caused by deletion of α-globin gene(s) resulting in 2 genotypes: – and -α for severe α-thalassemia 1 and mild α-thalassemia 2 forms, respectively. β-thalassemia, on the other hand, is mostly resulted from point mutations within and flanking structural β-globin gene which also gives rise to 2 sub-types which

are severe $\beta°$-thalassemia and mild β^+-thalassemia (Weatherall, 1983; Bernini and Harteveld, 1998; Thein, 1998;). Thalassemia is inherited in an autosomal recessive fashion, i.e., those homozygous or compound heterozygous for thalassemia alleles are clinically and hematologically affected, whereas those heterozygous or double heterozygous for thalassemia genes are clinically asymptomatic. In the latter group, however, some red blood cell parameters are modified and become significantly different from those in normal individuals (Tatu et al., 1997; Cao et al., 1998; Tatu et al., 1998; Tatu and Watcharasuwan, 2003). The altered values of red blood cell parameters are conventionally employed for discriminating between thalassemia heterozygotes and non-thalassemia individuals. The most conventionally-used ones include mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) (Ghosh et al., 1985; Rund et al., 1992; Jindadamrongwech et al., 1997; Harthoorn-Lasthuizen et al., 1998; Jaovisidha et al., 2000; Chan et al., 2001; Karimi and Rasekhi, 2002). MCV and MCH have been shown to be reduced and significantly lower in thalassemia heterozygotes than in non-thalassemia and have been successfully applied to screen thalassemia heterozygotes out of the non-thalassemia individuals.

Hypothetically, based on the molecular basis, those thalassemia heterozygotes bearing different molecular backgrounds could show different degrees of clinical and hematological severity, i.e., the more severe types of mutation, the more severely afftected. In the present study, we have determined the RBC count as well as other RBC indices in various thalassemia heterozygotes among Thai individuals. We could demonstrate the differences in RBC count among α-thalassemia 1 heterozygotes (SEA type), β-thalassemia heterozygotes and HbE heterozygotes. The findings were highly applicable in screening the thalassemia and hemoglobinopathies in general population.

MATERIALS AND METHODS

The study was conducted in 218 anonymous adult blood samples with Hb more than 13 g/dl in males and more than 12 g/dl in females, collected from the Hematology Laboratory Unit, Central Laboratory Department, Maharaj Nakorn Chiang Mai Hospital. Three ml of venous blood was collected and stored in EDTA. RBC parameters (RBC count, MCV, MCH, MCHC and RDW) were obtained by using an automated blood counter (Sysmex KX-21, Sysmex Corporation, Kobe, Japan) in all blood samples, followed by the evaluation for presence of hypochromic erythrocytes by the one-tube osmotic fragility test (OFT) (Tatu and Watcharasuwan, 2003). Those blood samples, positive for OFT, were subjected to the HbH inclusion body test for the detection of α-thalassemia 1 heterozygote (Tatu et al., 2003). Those positive for HbH inclusion body test were subsequently tested for β-thalassemia 1 (SEA type) allele by the Gap-PCR, using the method described previously (Sanguansermsri et al., 1999). The blood samples positive for OFT were also subjected to Hb identification by HPLC to detect heterozygous state of α-thalassemia and HbE hemoglobinopathy

(Primus, 1999). The data were finally analyzed using the statistical package to obtain descriptive statistics (mean and standard deviation), one-way analysis of variance (ANOVA), followed by the calculation of Least Significant Difference (LSD) and error bars were drawn to present the data graphically.

RESULTS

Based on the positive OFT results which were subsequently confirmed by the MCV values of less than 80 fl, 160 subjects were defined as normal individuals and 58 as thalassemia heterozygotes. The latter group consisted of 25 α-thalassemia 1 (SEA type) heterozygotes, 14 β-thalassemia heterozygotes and 19 Hb E heterozygotes. All the analysed RBC parameters were not different when compared between α-thalssemia 1 (SEA type) and β-thalassemia. However, RBC count and RDW in α-thalssemia 1 (SEA type) and β-thalassemia were significantly higher than those in Hb E heterozygote ($p < 0.05$). In addition, MCV, MCH and MCHC were significantly lower in α-thalssemia 1 (SEA type) and β-thalassemia than those in Hb E heterozygote ($p < 0.05$). Tables 1, 2 and Figure 1 summarize the results.

**Table 1.** Values of red blood cell parameters in the studied subjects.

	RBC (x10^6/µl)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	N
SEA	6.16±0.53	13.0±1.0	43.0±3.3	70.0±3.6	21.3±1.4	30.2±0.7	17.1±2.1	25
BT	6.21±0.63	12.7±1.2	42.5±3.5	68.7±5.4	20.6±2.1	30.0±1.4	17.6±2.6	14
ET	5.15±0.41	12.4±0.8	39.2±2.7	76.3±4.6	24.2±1.5	31.9±0.8	14.7±1.0	19
Normal	4.88±0.60	13.1±1.2	41.3±4.5	85.5±6.8	27.1±3.1	31.6±1.7	13.3±1.4	160

*SEA = α-thalassemia 1 heterozygote (SEA type), BT : β-thalassemia trait, ET : Hb E trait, Normal : Non-thalassemia + α-thalassemia 2 heterozygotes + some cases of HbE traits

Table 2. Levels of significance at 95% confidence interval for comparison of red blood cell parameters among different types of thalassemia heterozygote. This was firstly done by One-way ANOVA, followed by Fisherís Least Significant Difference (LSD) in case p-values by One-Way ANOVA was less than 0.05. [S = Significant, NS = Non-significant]

	ANOVA	SEA & BT*	SEA & ET*	BT & ET*
RBC (10^6/µl)	0.00 (S)	0.78 (NS)	0.00 (S)	0.00 (S)
MCV (fl)	0.00 (S)	0.52(NS)	0.00(S)	0.00 (S)
MCH (pg)	0.00 (S)	0.48 (NS)	0.00 (S)	0.00 (S)
MCHC (g/dl)	0.00 (S)	0.65 (NS)	0.00 (S)	0.00 (S)
RDW (%)	0.00 (S)	0.34 (NS)	0.00 (S)	0.00 (S)

Note: SEA = α-thalassemia 1 heterozygote (SEA type), BT : β-thalassemia trait, ET : Hb E trait, *Fisher's Least Significant Difference (LSD)
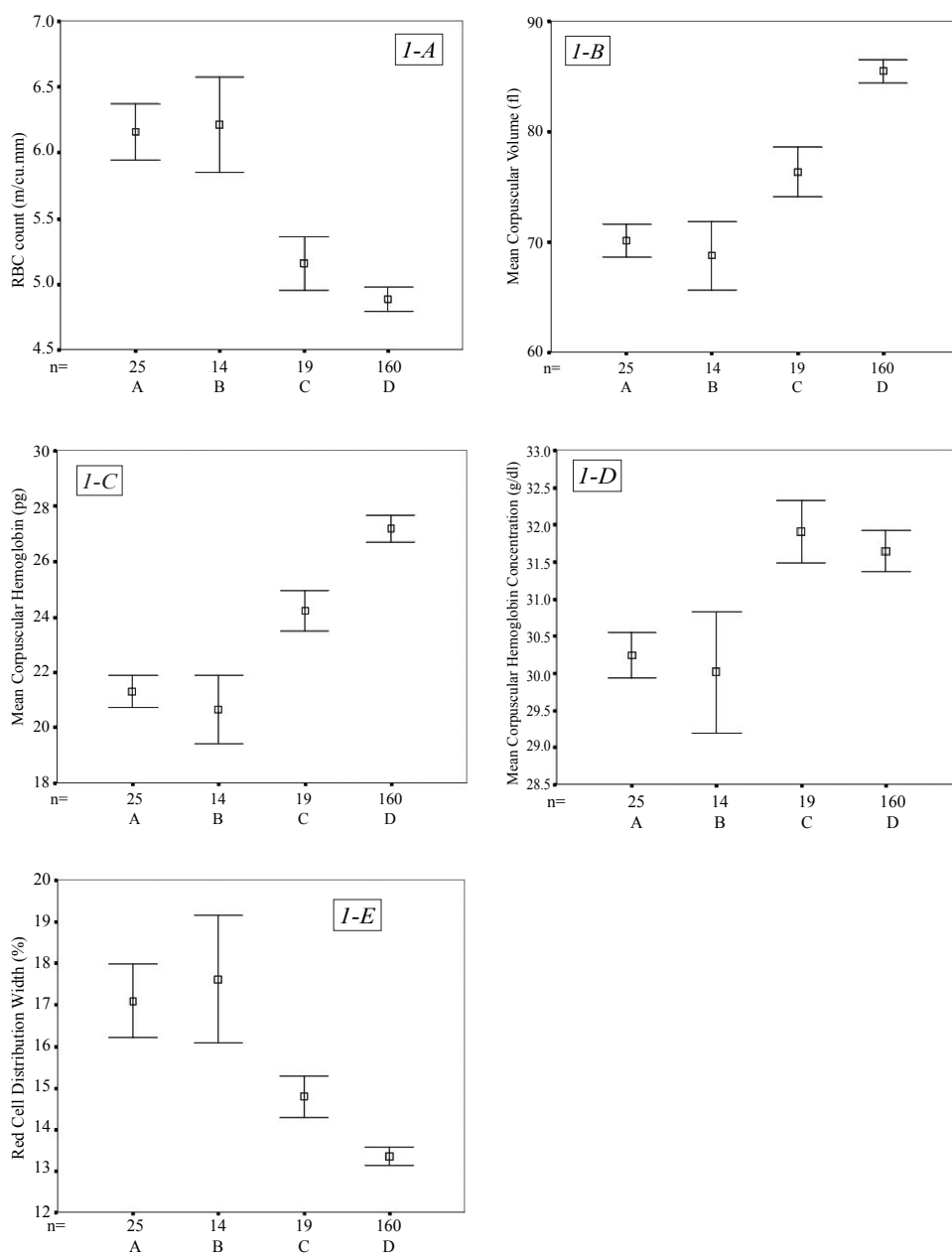
Figure 1. Comparisons of red blood cell parameters : RBC (1-A), MCV (1-B), MCH (1-C), MCHC (1-D), RDW (1-E) among the studied subjects : (A) = α-thalassemia 1 heterozygote (SEA type), (B)= β-thalassemia heterozygote, (C) = Hb E heterozygote, (D) = Non-thalassemia + α-thalassemia 2 heterozygote + some cases of HbE trait. Consult Table 2 for levels of significance.

DISCUSSION

RBC parameters including MCV, MCH, MCHC and RDW have been shown to be significantly different between normal individuals and thalassemia heterozygotes. MCV and MCH values are lower in the thalassemia heterozygotes than in normal. RDW, on the other hand, has been shown to be higher in thalassemia heterozygotes than in normal individuals and has been successfully implemented for thalassemia screening in some centers (Cesana et al., 1991; Villegas et al., 1998; Panyasai et al., 2002).

Among thalassemia heterozygotes, it has not been clear whether RBC count and other RBC parameters are different and of differentiation potentials between different types. In fact, the thalassemia encompasses two forms of its clinical outcome, i.e., mild and severe. This could lead to different degree of severity hematologically. In this study, we have determined red blood cell parameters including RBC count, red blood cell indicies (MCV, MCH and MCHC) and RDW in α-thalassemia 1 (SEA type), β- thalassemia and HbE heterozygotes diagnosed, using the standard criteria. The initial diagnosis of thalassemia heterozygote was based on the positive OFT and MCV value of less than 80 fl while those possessing negative OFT and MCV of more than 80 fl were classified as normal individuals. The authors were aware that some cases of HbE heterozygote were included in the so-called ìnormalî group. However, their RBC parameters were within normal limit and would be clearly distinguished from α- and β- thalassemia heterozygotes analysed in the study. Thus, this group of HbE heterozygote was ignored in the further analyses. The present study then concentrated on only α-thalassemia 1, β-thalassemia and HbE heterozygotes in which the alterations of RBC paramerters were evident. All of the analysed subjects were not anemic by the Hb concentration of more than 12 g/dl in females and more than 13 g/dl in males following the criterion set by WHO (Skjelbakken et al., 2005; Atti et al., 2006). Types of thalassemia among these heterozygotes were determined by the standard techniques such as HbH inclusion body test, followed by Gap-PCR for α-thalassemia 1 heterozygote (SEA type), Hb identification by HPLC for HbA_2/E quantification for β-thalassemia and HbE heterozygotes. From our previous work, we have shown that by using the modified BCB technique for demonstration of intra-erythrocytic Hb H inclusion body test, most of those positive for this test were α-thalassemia 1 heterozygote of SEA types and some that were not positive for SEA type were presumed to be either homozygous α-thalassemia 2 or α-thalassemia 1 of other deletional or mutation types (Tatu et al., 2003). This study, however, also demonstrated the same findings as those generated by the previous work (data not shown), implicating that not all subjects, positive for HbH inclusion body test, were α-thalassemia 1 heterozygote of SEA type. This has left the possibility that there may be other types of α-thalassemia 1 or some homozygote for α-thalassemia 2 in these subjects. Further work needs to be done to clarify this question.

As hypothesised, the RBC parameters in thalassemia heterozygotes of severe types such as α-thalassemia 1 (SEA type) and β-thalassemia (mainly the codons 41/42 (-TTCT), codon 17 (A-T), IVS-I-nt1 (G-T) and codons 71/72 (+A) of the β-globin gene, according to the published data (Sirichotiyakul et al., 2003) were significantly

different from heterozygotes of mild form of hemoglobinopathy (Hb E). RBC count and RDW were higher but MCV, MCH and MCHC were lower in severe form than mild form of these heterozygotes. This evidently indicated that erythropoiesis was more adversely affected in α-thalassemia 1 (SEA type) and β-thalassemia than in HbE. RBC count as well as MCV, MCH and RDW were the RBC parameters that were significantly modified. This study showed that these RBC parameters could differentiate between severe and mild form of thalassemia in heterozygote. However, these RBC parameters still were not of differentiating application among severe types of thalassemia heterozygotes, i.e., α-thalassemia 1 (SEA type) and β-thalas- semia. This particular finding was discordant with that previously shown by Han et al., (1990) which showed the differences of the RBC parameter (MCV) between α-thalassemia and β-thalassemia heterozygotes. However, Han and colleague's study did not perform the sub-typing of the thalassemia in the heterozygotes into mild and severe forms. Most interestingly, the study conducted by Jindadamrongwech et al., (1997) showed that the RBC parameters such as MCV and RDW could be used to screen α-thalassemia 1 heterozygote or homozygous α-thalassemia 2. Both of those conditions are severe types of α-thalassemia. The finding in the present study added to the information that besides MCV, MCH, MCHC and RDW, the RBC count could also be used to screen α-thalassemia (SEA type) and/or β-thalassemia out of the mild form of thalassemia in heterozygous state.

Although no statistical analysis was peformed, it was obvious that the values of red cell indices were lower and RBC count as well as RDW were higher in α-, β- and HbE heterozygotes than in normal individuals. This phenomenon was not surprising as it had already been documented by several studies. The normal individuals were recruited in this study only to validate the normal values of the red cell parameters and to ensure the abnormal values that would be found in the thalassemia heterozygotes.

In conclusion, we have successfully demonstrated that the RBC count was not applicable in distinguishing heterozygous α-thalassemia (SEA type) from hetero- zygous β-thalassemia. However, it has the potential in differentiating heterozygous HbE from heterozygotes of those two severe forms of thalassemia, particularly when considered together with MCV, MCH and RDW.

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