EVALUATION OF RED BLOOD CELL INDICES RELATED DISORDERS AMONG ELIGIBLE BLOOD DONORS AT THE UNIVERSITI PUTRA MALAYSIA (UPM)

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Abstract. Pre-donation screening declarations and hemoglobin (Hb) testing are measures used to determine the quality of donated blood. The copper sulphate ($CuSo_4$) method used to screen for blood abnormalities can give inaccurate results if strict quality control is not applied. Blood donors who are carriers of thalassemia and those with mild iron deficiency anemia (IDA) are usually asymptomatic and frequently missed at blood donation. The aim of this study was to evaluate the red blood cell (RBC) indices related disorders among blood donors who were deemed qualified to donate blood after screening with $CuSo_4$ method. One hundred fifty-eight volunteer blood donors at the Universiti Putra Malaysia (UPM), who had passed the $CuSo_4$ screening method, were recruited for this study. Their bloods specimens were examined with a complete blood count. Subjects with a low mean corpuscular hemoglobin (MCH) level were examined further by checking a serum ferritin level, Hb quantification, and molecular analysis to examine for common RBC disorders. Fourteen point six percent of subjects had a low Hb level, two (1.3%) had IDA and four (2.5%) had thalassemia or some other hemoglobinopathy. Using a MCH level <27 pg as a cut-off point, 58 subjects (36.7%) had suspected IDA, thalassemia or some other hemoglobinopathy. Eight point nine percent of subjects with a normal Hb level had thalassemia, and 3.8% had IDA. Malaysia has a high prevalence of thalassemia and other hemoglobinopathies. Pre-donation accurate screening is crucial to protect the quality of blood transfusion products. Public education regarding RBC disorders especially among blood donors is important.

Keywords: blood donors, copper sulphate, thalassemia

INTRODUCTION

The main goal of blood donation is to provide good quality blood and blood components. To achieve this, proper selection of donors is mandatory (Whitsett *et al*, 2012). The copper sulphate ($CuSo_4$) method is a pre-donation test used to determine if the blood meets the minimum hemoglobin (Hb) requirement. Some studies have found that the $CuSo_4$ method is not sensitive for detecting anemia (Sawant *et al*, 2007; Tondon *et al*, 2009; Garg *et al*, 2012).

Iron deficiency anemia(IDA) and thalassemia are common red blood cell (RBC) disorders (Angastiniotis and Modell, 1998). In Malaysia, the prevalences of IDA

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and thalassemia trait are 3.5% and 8.25%, respectively (Azma *et al*, 2012).

RBC transfusions are indicated to provide enough oxygen transport media to meet tissues demands. The oxygen carrying capacity depends on the concentration of Hb in the blood and the half life of the RBC. Donor selection is important to reduce the chances of obtaining blood from a donor with a blood problem.

The $CuSo_4$ test is the primary Hb screening method used in Malaysia (Nadarajan and Eow, 2002). We conducted this study to determine the prevalence of RBC indices related disorders among donors whose blood passed the $CuSo_4$ screening test.

MATERIALS AND METHODS

Ethical clearance

This study was approved by National Medical Research Register and ethics committee of the Faculty of Medicine and Health Science, Universiti Putra Malaysia (UPM).

Study population

One hundred fifty-eight subjects were included in the study. Each of them were determined to be eligible blood donors based on the guidelines of the National Blood Centre (NBC). Donors filled out a form about health and behaviors. Their Hb level was then estimated using the $CuSo_4$ method and they were interviewed by an attending medical officer. Informed consent was obtained from each subject prior to providing a blood sample.

Sample collection

Four milliliters of blood was obtained from each subject. Three milliliters was collected in a tube containing ethylenediaminetetraacetic acid (EDTA) and was used to perform a complete blood count (CBC), Hb analysis (Hb subtype quantification by HPLC) and molecular study. One milliliter of blood was collected in a plain tube and examined for serum ferritin level. The CBC was conducted using a SYSMEX KX21 (Block Scientific, Bellport, NY) machine. Subjects with a MCH <27 pg were classified as having hypochromic RBC indices. A low level of MCH is a common indicator of IDA and thalassemia (Fialon *et al*, 1993). The serum ferritin level was obtained using a Architect ci8200 analyzer (Abbott Diagnostic, Abbott Park, IL) following manufacturer's instructions. A serum ferritin level <10 ng/dl was considered to be IDA. Hb subtypes quantification for suspected β-thalassemia variant disorders was screened using a Biorad Variant 2 machine (Biorad Laboratory, Hercules, CA). A Hb A_2 percentage of 3.5% - 9% was suggestive of β-thalassemia trait. A Hb A_2 percentage >10% was considered indicative of some other β-variant disorder (Fucharoen *et al*, 1998; Colah *et al*, 2007). Samples with a low MCH, a normal serum ferritin level and normal Hb A_2 and Hb F percentages were suspected of having α-thalassemia trait (Harteveld and Higgs, 2010). These samples were subjected to molecular analysis. Single tube multiplex polymerase chain reaction (PCR) was used to detect α-thalassemia (Chong *et al*, 2000). This is a rapid, easy technique to diagnose α-thalassemia mutations ($\alpha^{3.7}$-thalassemia, α^{SEA}-thalassemia, $\alpha^{4.2}$-thalassemia, α^{FIL}-thalassemia and α^{THAI}-thalassemia). The PCR tube contained 12.5 µl of 2X HotStar Taq DNA polymerase (QIAGEN, Hilden, Germany) (10X PCR buffer, 5X Q-solution, 25 mM $MgCl_2$), 2.5 µl of 5X Q-solution, 1 µl of 100 ng of genomic DNA and 16 different primer pairs at various concentrations. The primers used were: 0.5 µl of 2 µM of A2-F/3.7-F, 20.5-F, A2-R, 3.7-R/20.5R, SEA-F, SEA-R, MED-F, MED-R primers, 0.75 µl

Table 1
Thalassemia or other hemoglobinopathy among study subjects.

RBC parameters	No.
Total blood donors	158
Gender (male/female)	71/87
Number of Hb <12.5 g/dl donors	23
IDA	2
Thalassemia or other hemoglobinopathy(α/β/E)	(4/0/0)
Number of donors with MCH <27 pg	58
IDA	8
Thalassemia-hemoglobinopathies	18
Gender (female/male)	10/8
Mean age (years)	23.5 (21-27)
Mean Hb (g/dl)	12.6 (9.2-15.2)
α- thalassemia trait type	15
$\alpha^{3.7}$- thalassemia trait	12
$\alpha^{4.2}$- thalassemia trait	2
α^{SEA}- thalassemia trait	1
β-thalassemia	0
Hb E trait	3

of 3 µM of THAI-F, THAI-R primers, 1.25 µl of 5 µM of 4.2-F, 4.2-R, LIS1-F, LIS1-R primers and 2.5 µl of 10 µM of FIL-F, FIL-R primers. Finally, an appropriate volume of sterile distilled water was added to each microfuge tube to obtain a total volume of 25 µl.

The PCR was conducted as follows: an initial denaturation for 15 minutes at 95°C for one cycle followed by 32 cycles at 98°C for 45 seconds, 61.5°C for 90 seconds for annealing, 72°C for 135 seconds for extension followed by 5 minutes at 72°C for the final extension. The PCR amplicons were electrophoresed with 1.5% agarose gel and visualized using an UV transil-luminator.

RESULTS

One hundred fifty- eight subjects participated in this study, 87 were female and their ages ranged from 19 to 55 years old. Sixty-four point five percent of the subjects were Malay, 31.1% were Chinese and 4.4% were Indian. The mean Hb level was 13.2 (range 9 - 20.4) g/dl.

Twenty-three subjects (14.5%) had a low Hb (<12.5 g/dl for males (n=5) and <11.5 g/dl for females (n=18), even though the $CuSo_4$ test result for them was normal. Of these 23 subjects with a low Hb, two had IDA and four had thalassemia or some other hemoglobinopathies. Based on a MCH cut-off level of <27 pg, 58 subjects (36.7%) had either IDA or thalassemia. The mean hemoglobin in these 58 subjects was 12.8 g/dl. Serum ferritin level revealed 8 subjects (5.1%) had IDA (respondents with low Hb and low serum ferritin level) and six of them with latent IDA (respondents with normal Hb and low serum ferritin level).

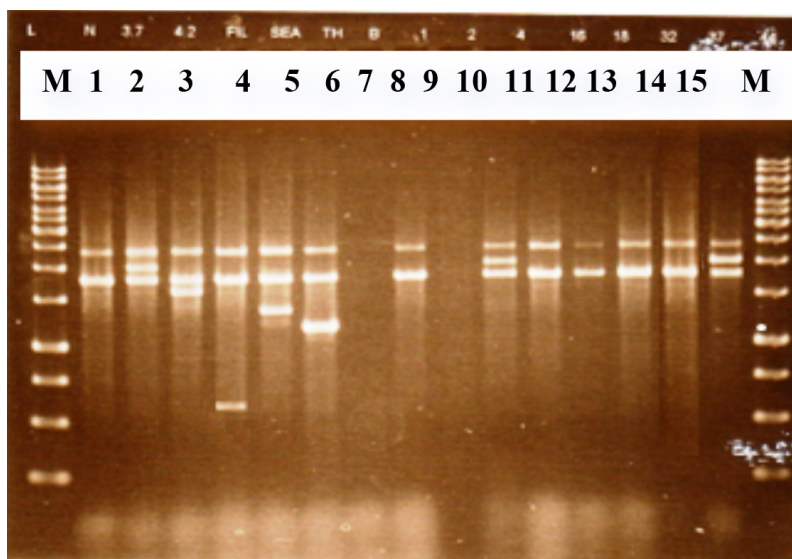Hb quantification by HPLC was normal in 155 of the 158 subjects (98.1%). Three

Fig 1–Single tube multiplex PCR results of subjects with α-thalassemia trait. Lane M, 1 kb DNA ladder; lane 1, normal (αα/αα); lane 2, heterozygus α3.7-thalassemia (-α3.7/αα); lane 3: heterozygus α4.2-thalassaemia (-α4.2/αα); lane 4: heterozygus αFIL-thalassemia (--FIL/αα); lane 5, heterozygus αSEA-thalassemia (--SEA/αα); lane 6, heterozygus αTHAI-thalassaemia (--THAI/αα); lane 7: blank; lane 8 to 15: α-thalassemia suspected samples.

subjects (1.9%) had Hb E trait. No cases of β-thalassemia trait were identified (Table 1). DNA analysis revealed α-thalassemia in 15 subjects (9.5%). Two subjects (1.3%) had α-thalassemia trait with IDA.

Three types of α-thalassemia were detected ($\alpha^{3.7}$, $\alpha^{4.2}$, α^{SEA}). Twelve subjects (7.5%) had $\alpha^{3.7}$-thalassemia, two subjects (1.3%) had $\alpha^{4.2}$-thalassemia and one subject (0.6%) ha α^{SEA}-thalassemia (Fig 1). Eighteen subjects (11.4%) had either thalassemia or some other hemoglobinopathy (3 with Hb E trait and 15 with α-thalassemia trait). The other subjects with a low MCH probably had other type of thalassemia or hemoglobinopathies which cannot be identified in this study.

DISCUSSION

Correctly selecting blood donors is important. The $CuSo_4$ method is an easy, inexpensive method to screen for Hb abnormalities (*ie*, anemic status) among donors. However, it has a poor sensitivity for detecting thalassemia, other hemoglobinopathies and IDA. In our study, 23 subjects (14.6%) passed the $CuSo_4$ test even though they have low Hb level (anemia). Our findings are similar to those by Nadarajan *et al* (2008), who found 21.3% of Malaysian subjects passed the $CuSo_4$ test even though their Hb levels were <12.5 g/dl. Tondon *et al* (2009), found 6.9% of Indian subjects passed the $CuSo_4$ test even though they had a Hb abnormality. However, the authors rejected 7.9% of blood donors with normal Hb level which was reconfirmed by FBC analyzer. The study of Tendon *et al* (2009) concluded that the sensitivity and specificity of the $CuSo_4$ screening method in their study were 98.8% and 58.1%, respectively. Sawant *et al* (2007) found 29% of blood donors in India

who were screened by $CuSO_4$ method were rejected from donation even though their Hb was >12.5 g/dl.

HemoCue is an another alternative method for Hb estimation level for predonation screening (specificity 84.4% and sensitivity 99.4%). Financial constraints however limit its usage (Tondon *et al*, 2009). The hemoglobin color scale (HCS) method is the least accurate method for Hb screening methods in blood donors with many errors (specificity 81.8% and sensitivity 87.3%) and thus not suitable for pre-donation screening (Tondon *et al*, 2009). Overall, none of these available techniques are the ideal method for Hb estimation (Tondon *et al*, 2009). The Canadian Blood Services uses a two-step method to screen for Hb problems; the $CuSO_4$, and the HemoCue tests (Goldman *et al*, 2012). They also, recommended a spectrophotometer (DiaSpect system) to screen for Hb problems.

Important goals of a national blood program are to supply safe, adequate blood and blood products. IDA, thalassemia and other hemoglobinopathies in blood donors affect the quality of donated blood. After receiving blood from a person with a hemoglobinopathy, 1%-14% abnormal RBC were detected in the recipient in one study (Kozarski *et al*, 2006). Gupta *et al* (2011) reported that Hb S ranging from 9.9% to 18.5% could be detected in post-transfusion of young recipients with β-thalassemia major who recieved blood from a person with HbS. The transfusion reaction from blood component such as fresh frozen plasma, and platelet concentrates is more likely to happen if a donor has thalassemia or some other hemoglobinopathies (Rosline *et al*, 2006).

RBCs of thalassemia or other hemoglobinopathy are not considered equivalent to normal RBCs and should not be given to patients whose blood oxygen might be compromised (Josephson *et al*, 2007). The problem of receiving blood from thalassemia or other hemoglopathies donors is more prominent when the recipients are children with the low birth weight (Rosline *et al*, 2006). Beside all of the mentioned side effects for recipient, blood donation by annemic donor can adversely affect the donor themselves. Blood donation may cause iron depletion in donors with IDA and treatment of it requires financial resources and has an adverse effect on social, economic and psychological behaviors (Javadzadeh Shahshahani *et al*, 2005).

Rosline *et al* (2006) studied thalassemia and other hemoglobinopathies at a blood donation program at the University Science Malaysia (USM), she found 16.3% of donors had thalassemia or other hemoglobinopathies; 11.3% had Hb E trait and 5% had β-thalassemia trait. Eleven point four percent of donors in our study had thalassemia or other hemoglobinopathies; 1.9% had Hb E trait and 9.5% had α-thalassemia trait. None of the donors in our study has β-thalassemia trait. This finding may not reflect the current prevalence of thalassemia and IDA among Malaysian community donors. Furthermore, the study of Rosline *et al* (2006) and our study were focusing at two different donor populations with different background.

Malaysia has high prevalence of thalassemia or other hemoglobinopathies of 4.5% (Quek *et al*, 2007). Proper pre-donation screening and counseling to increase awareness of these disorders is important to minimize the risk for people with IDA, thalassemia and other hemoglobinopies to donate blood.

National policies for blood pre-donation screening vary by country. Public education is important to reduce the chance of a donor with thalassemia or other hemoglobinopathies donating blood. A thalassemia screening program could be incorporated into the blood donation program of Malaysia. This could reduce the number of people with thalassemia or other hemoglobinopathies donating blood. Choosing an appropriate pre-donation screening method is important for the quality of donated blood, and cost of program.

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