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Research Article

Thalassemia Carriers among Healthy Blood Donors

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ABSTRACT

Thalassemia is a common hereditary anaemia in Southeast Asia which may also be found among healthy blood donors. The established screening method is less sensitive and can miss donors with clinically silent Thalassemia. The Thalassemia screening tests may help in selection of truly healthy blood donors, hence providing the most functional red cell concentrates for blood transfusion. This is an extension study from our previous work, aiming to detect thalassemia trait among clinically asymptomatic blood donors. We performed Thalassemia screening in 738 healthy blood donors who were allowed for blood donation using full blood count and haemoglobin (Hb) analysis. After screening analyses using high performance liquid chromatography, capillary zone electrophoresis and agarose gel electrophoresis, 85 samples were excluded from the study due to inadequate volume for DNA analysis. Five hundred twelve (512/653; 78.4%) samples were within normal limit and 74 (74/653; 11.3%) samples with Hb less than 12.5 g/dl. Thirty eight donors were found to have Thalassemia and/or haemoglobinopathies by Hb analysis. The remaining 105 blood donors samples with value of MCV less than 80 fl and/or MCH lower than 27 pg with no detectable abnormal pattern from Hb analysis subjected to multiplex PCR that was capable of detecting deletional and nondeletional of the α-globin gene. Majority (79/653; 2.1%) of them had no identified mutation while 23 (23/653; 3.5%) of them had heterozygous α-3.7 deletion, 2 (2/653; 0.3%) had heterozygous α-SEA deletion and only one (1/653; 0.1%) had heterozygous α-4.2 deletion. This data indicates that 74 (74/653; 11.3%) of our blood donors were anaemic, 64 (64/65; 39.8%) had haemoglobinopathies and 79 (79/653; 12.1%) were presumptively had iron deficiency anaemia based on red blood cell indices. Provision of best quality pack red cells should be selected for patients who require regular blood transfusion in order to maintain appropriate Hb level.

Keywords: Thalassemia trait; Asymptomatic blood donors; Effect of transfusing thalassemic blood

INTRODUCTION

Haemoglobinoapathies and Thalassemia are common in South East Asia countries. It is incurable but it can be prevented by blood screening, genetic counseling for married couples or partners and antenatal diagnosis during pregnancy. Many studies have shown small frequencies of Thalassemia carriers among blood donors [1-3]. The established screening method is less sensitive in detecting Thalassemia among blood donors with clinically silent Thalassemia. Therefore the Thalassemia screening tests may help in selection of suitable blood donors, hence providing the most functional red cell concentrates for blood transfusion. The main objective of blood transfusion is to restore adequate oxygen supply to the recipient by achievement of appropriate haemoglobin level. This is an extension study from our previous work, which was reported in 2014 [4]. The aim of the study was to detect thalassemia trait among clinically asymptomatic blood donors.

METHODS

Sample collection

A total of 738 samples from Malaysian blood donors were collected from Tengku Ampuan Rahimah Hospital for about four months period. Those who passed all the standard criteria for blood donation as elaborated in our previous report [4] were selected

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for the study. Thalassemia screening tests were performed on all samples and DNA analysis for alpha Thalassemia was performed for some samples with suggestive diagnosis of alpha Thalassemia. Consents were obtained from each of blood donor prior to the blood taking and they had been explained about the purpose of the study.

Laboratory test methods

Full blood count was determined by an automated haematology analyzer, Sysmex XE-2100D and Sysmex XE-2100 Alpha (Sysmex, Japan) within four hours. Haemoglobin (Hb) analysis was performed using three methods; high performance liquid chromatography (HPLC), agarose gel electrophoresis and capillary zone electrophoresis (CE). HPLC was performed using Bio–Rad VARIANT II Haemoglobin Testing System (Hercules, USA) and CE technique was performed using the Sebia Capillarys system (Sebia, France). Agarose gel electrophoresis using Sebia Hydrasis (Sebia, France) at alkaline pH 8.6 was only run on samples with detected Hb variants on HPLC and CE. From this method, the resulting electropherograms were evaluated visually for pattern abnormalities. The recommended normal ranges for Hb subtypes by HPLC for healthy adults were as follows: Hb A, more than 90%; Hb F, less than 1%; and Hb A2, 2.2% to 3.2%. All of haemoglobinopathy cases that can be detected by the screening methods were not processed for DNA studies although it is the definitive method to confirm the findings. Cases with MCV less than 80 fl, MCH lower than 27 pg and no detected abnormal pattern in three methods of Hb analysis only were subjected for DNA analysis. DNA studies for α-globin gene were performed on extracted DNA using in-house multiplex PCR analysis. This method is capable of detecting single (−α$^{3.7}$, −α$^{4.2}$) and double α-globin gene deletion ($--^{SEA}$, $--^{FIL}$, $--^{MED}$, $--^{20.5}$ and $--^{THAI}$) as well as point mutation of the α-globin gene [initiation codon (ATG→A_G), codon 30 (ΔGAG), codon 35 (TCC→CCG)/Hb Quang Sze and termination codon (TAA→CAA)/Hb Constant Spring].

RESULTS

Out of 738 samples, 85 samples were excluded from the study since the samples were inadequate for DNA analysis. Five hundred twelve (512/653; 78.4%) samples were within normal limits and 74 (74/653; 11.3%) samples with Hb less than 12.5 g/dl; whose four (4/74; 5.4%) of them had haemoglobinopathies. Low mean corpuscular haemoglobin value (MCH less than 27 pg) was observed in 225 (225/653; 34.5%) donors, of whom 37 (37/225; 16.4%) had haemoglobinopathies. Haemoglobinopathy was found in one (1/653; 0.1%) donor with normal MCH (28.2 pg) and low mean corpuscular volume value (MCV less than 80 fl). From a total of 38 (38/653; 5.8%) donors with haemoglobinopathies, 19 (19/653; 2.9%) were Hb E trait, six (6/653; 0.9%) were Hb E with β-Thalassemia co-inheritance, five (5/653; 0.7%) were β-Thalassemia trait, two (2/653; 0.3%) were Hb A2 prime, one (1/653; 0.1%) was Hb Lepore and one (1/653; 0.2%) was alpha chain variant. There were four (4/653; 6.1%) donors with Hb Constant Spring, two of them were detected to have Hb Constant Spring only by using two methods, ie. CE and alkaline gel electrophoresis, which was in contrast to our previous report [4]. From the initial number (738) of collected blood samples, 190 (190/738; 25.7%) blood donors had the possibility of alpha Thalassemia since they had low MCV and/or MCH with no detected abnormal pattern from Hb analysis. However DNA analysis of the alpha globin gene was only performed in 105 (105/653; 16.1%) samples since 85 of the samples were inadequate for DNA analysis. We found 23 (23/653; 3.5%) donors with heterozygous −α$^{3.7}$ deletion, two (2/653; 0.3%) cases with heterozygous --SEA deletion and one (1/653; 0.1%) case with heterozygous −α$^{4.2}$ deletion. Majority of the blood donors with heterozygous α-3.7 deletion were Malay (13/23; 56.5%), followed by Indian (8/23; 34.8%), Chinese (1/23; 4.3%) and other ethnicity (1/23; 4.3%)]. Both of the donors with heterozygous α--SEA deletion were Chinese. The value for their MCV was 64.5 to 81.0 fl and MCH was 19.7 to 26.2 pg, while the MCV value and MCH value for a donor with −α$^{4.2}$ deletion was 77.9 fl and 25.0 pg. The haematological parameters for heterozygous −α$^{3.7}$ deletion are shown in Table 1. The remaining of 79 (79/653; 12.1%) donors had no identified mutation and they might have iron deficiency anaemia. Although serum iron profile was not performed to confirm this finding, the examination of the peripheral blood smear found that there were features of iron deficiency in the red cells of these donors.

Table 1: Haematological parameters for heterozygous −α$^{3.7}$ deletion in blood donors (values are expressed as mean and standard deviation).

Blood Indices	Heterozygous α-3.7 deletion [mean(SD)]	
n	23	-
Hb (g/dl)	13.9 (1.3)	-
RBC (1012/l)	5.5 (0.4)	-
MCV (fl)	80.03 (25.1)	-
MCH (pg)	25.1 (1.2)	-
MCHC (g/dl)	31.4 (1.2)	-
Hb A (%) HPLC/CE	86.3 (4.0)	97.6 (0.4)
Hb A2 (%) HPLC/CE	2.6 (0.2)	2.3 (0.2)
Hb F (%) HPLC/CE	0.4 (1.0)	0.0 (0.0)

Hb: Haemoglobin; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration

DISCUSSION

Selection of suitable blood donors is important to safeguard their health and to provide good quality blood to the recipients. Based on the established criteria, the blood donors are screened through brief medical history, observation and simple tests such as copper sulphate gravimetric test. The Hb is considered normal if the donors pass the gravimetric test. However this test has poor sensitivity for detection of early stage of iron deficiency anaemia and haemoglobinopathies. This is in accordance with the previous studies that had reported about 14 to 20% of the blood donors who passed the gravimetric test were actually had microcytic hypochromic anaemia when tested with haematology analyser [1-3]. In our study, 11.3% (74/653) of the anaemic (Hb<12.5 g/dL), 9.8% (64/653) blood donors were carriers for Thalassemia and haemoglobinopathies; and 12.1% (79/653) were presumptively had iron deficiency anaemia based on blood counts and red cell morphology. A study by Tondon et al. [5] had concluded that the sensitivity and specificity of the copper sulphate gravimetric test was 98.8% and 58.1%; which explained why both disorders were easily missed. Thalassemia screening activities have been carried out in Malaysia since 2004. Currently the main group for screening and genotype confirmation is the 16-year old adolescent. These programs are beneficial and more feasible to be carried out among blood donors. Nevertheless the cost implication should be taken into account prior to formulating the national policy.

It is a common practice worldwide to accept blood from thalassemic carrier donors who meet the minimum Hb level for blood donation [6]. However, the exception is not applied to Hb S carrier; neither apheresis nor whole blood donations are allowed. The blood is not suitable for intrauterine transfusion or neonatal exchange transfusion. It may also exacerbate the red cells sickling in those with sickle cell disease which ultimately cause sickling crisis [6]. Countries in Southeast Asia have extremely high prevalence of haemoglobin E; the frequency is widely varies from 3 to 60% [7-9]. The incidence of Hb E among our blood donors is 3.9%, while in Thailand is 5.2% [10]. Hb E is unstable haemoglobin and has been shown to have increased sensitivity to oxidants leading to shorten red cell survival [11]. It was shown that 25 to 50% of transfused thalassemic cells were destroyed within 10 to 20 days rendering the transfusion relatively ineffective [12]. Since the prevalence of transfusion dependent beta Thalassemia major patient is also high in Southeast Asia, the selection of high quality pack red cells should be considered.

CONCLUSION

Thalassemia is a growing global public health problem which needed to be addressed seriously for control and prevention. Thalassemia screening among blood donors can be one of the strategies as it can prevent the risk of donors giving birth to babies with severe hemoglobinopathies. Public awareness about carrier screening is also important in order to reduce the number of blood donors with Thalassemia and haemoglobinopathies from donating blood. A local study for assessing the effect of transfusing thalassemic blood in patients is required before considering Thalassemia screening test in blood donors.

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CONFLICT OF INTEREST

The authors have no conflict of interests to disclose.

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