Thalassaemia Screening among Healthy Blood Donors in Hospital Tengku Ampuan Rahimah, Klang

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ABSTRACT

Program saringan talasemia telah diwujudkan di Malaysia semenjak tahun 2004. Program ini bertujuan untuk mengurangkan beban akibat penyakit ini dengan mengenal-pasti pembawa talasemia. Walaubagaimanapun, sambutan terhadap aktiviti saringan ini didapati kurang memuaskan kerana kurangnya kesedaran orang ramai akan kepentingan saringan talasemia. Kaedah alternatif adalah menyaring penderma darah. Matlamat kajian ini adalah untuk mengetahui prevalens pembawa talasemia di kalangan penderma darah yang sihat. Tujuh ratus tiga puluh lapan penderma darah yang sihat telah disaring di Hospital Tengku Ampuan Rahimah, Klang menggunakan kaedah cation-exchange high performance liquid chromatography (HPLC) dari Julai hingga September 2010. Semua kes varian hemoglobin seterusnya diproses menggunakan gel electrophoresis pada pH alkali. Hasil kajian menunjukkan penderma darah terdiri daripada bangsa Melayu 413 (56%), India 162 (22%), Cina 148 (20%) and lain-lain 15 (2%). Terdapat 19 (2.6%) trait hemoglobin E, enam (0.8%) hemoglobin E/talasemia-α, dan lima (0.7%) trait talasemia-β. Hemoglobin Constant Spring dan hemoglobin A₂ prime dilihat dalam dua (0.3%) kes; dan Hemoglobin Lepore serta varian rantaian alpha dalam satu (0.2%) kes. Walaubagaimanapun talasemia-α dan talasemia-β hemoglobin A₂ normal tidak boleh diabaikan dalam 190 (26%) kes dan memerlukan analisa DNA untuk pengenalpastian jenis talasemia tersebut. Saringan talasemia ke atas...
Thalassaemia screening programme has been conducted in Malaysia since 2004. The aim of the programme was to reduce the burden of the disease by identifying thalassaemia carriers. However, the response towards the screening activities was unsatisfactory as there was lack of public awareness against the importance of thalassaemia screening. An alternative approach is to screen blood donors. The purpose of this study was to observe the prevalence of thalassaemia carriers among healthy blood donors. Seven hundred and thirty eight healthy blood donors were screened in Hospital Tengku Ampuan Rahimah, Klang from July to September 2010 using cation-exchange high performance liquid chromatography (HPLC). Cases with haemoglobin variants were further analyzed by gel electrophoresis at alkaline pH. Result shows that the blood donors consisted of 413 Malays (56%), 162 Indians (22%), 148 Chinese (20%) and 15 others (2%). There were 19 (2.6%) individuals with haemoglobin E trait, six (0.8%) with co-inheritance of haemoglobin E and α-thalassaemia and five (0.7%) with β-thalassaemia trait. Haemoglobin Constant Spring and haemoglobin A₂ prime were observed in two (0.3%); and Haemoglobin Lepore and alpha chain variant in one (0.2%). α-thalassaemia and normal haemoglobin A₂ β-thalassaemia could not be excluded in 190 cases (26%), as they required deoxyribonucleic acid (DNA) studies for identification. Thalassaemia screening in blood donors is more feasible and effective. Therefore, a wider scale population screening including blood donors could benefit the existing thalassaemia screening programme in Malaysia.

Keywords: thalassaemia screening, blood donors, haemoglobin variants, Malaysia

INTRODUCTION

Thalassaemia and haemoglobinopathies not only result in serious health problem, but also creates a heavy economic burden to the country (George 2001). Therefore, many countries including Malaysia have established thalassaemia screening programmes (HKCOG 2003; Samavat & Modell et al. 2004; MOH 2009) in order to identify carriers. These programmes mainly focussed on couples of childbearing age either before or during pregnancy. It was also offered as a premarital screening in order to help carriers to make informed decision on the choice of marital partner, since marriage between carriers is not encouraged. Since 2004, Malaysia has carried out screening activities through two different approaches on volunteer participants. In cascade screening, the
close relatives of the patients would be tested, while in target screening, it may involve expecting mothers, adolescents in schools and colleges, and also those who are attending the government health care centres (MOH 2009). However, even though the screening for thalassaemia is free of charge, the response rate is poor in our nation due to lack of awareness, especially in lower educated individuals (Anjanna et al. 2011; Wong et al. 2011).

While campaigns are consistently carried out to improve public awareness and to encourage target groups to participate in voluntary thalassaemia screening, an alternative and a feasible approach would be to screen blood donors. In our practice, the blood donors are clinically assessed for medical illnesses through standard questionnaires and simple physical examinations. The pre-donation haemoglobin level is also measured with minimal cut-off of 12.5 g/dL as the acceptance criteria. Prospective donors who are healthy with haemoglobin levels within the normal range are permitted for blood donation. In order to prevent the adverse effect of blood donation to the donors as well as the recipients, the donated blood is always screened for transfusion-transmissible infections. Regarding these sequences of donor selection and screening tests, we might miss donors with thalassaemia trait because carriers of thalassaemia are asymptomatic in general and their haemoglobin values are sometimes above the minimal requirement (Bryant et al. 2009). In the present study, thalassaemia screening was carried out on post-donated blood to find out the prevalence of thalassaemia carrier among healthy blood donors.

MATERIALS AND METHODS
A cross-sectional study was conducted on routine diagnostic samples sent for thalassaemia screening in Institute for Medical Research. A total of 738 samples from Malaysian blood donors were received from Tengku Ampuan Rahimah Hospital from July until September 2010. The donors were explained about the purpose of the study and consent were obtained from each of them prior to the blood taking. They were selected according to the standard criteria prepared by the hospital including age between 18 and 65 years old, minimum weight of 45 kg and minimum of five hours sleep. Healthy donors with acceptable haemoglobin level were allowed to donate when the time interval from the last donation was at least eight weeks. The acceptable haemoglobin level was presumed ranging from 12.5 to 18.0 g/dl if the donors passed the copper sulphate gravimetric test.

Five ml of venous blood specimen were collected in ethylenediaminetetraacetic acid (EDTA) tube during the blood donation process. Full blood count was performed by an automated haematology analyzer, Sysmex XE-2100D and Sysmex XE-2100 Alpha (Sysmex, Japan) in the hospital on the day of collection, while further analyses were conducted in Institute for Medical Research on the next day (within 48 hours). We used Bio–Rad VARIANT II Haemoglobin Testing System (Hercules, USA), a
fully automated cation-exchange high performance liquid chromatography (HPLC) for haemoglobin analysis. Agarose gel electrophoresis using Sebia Hydrasis (Sebia, France) at alkaline pH (8.6) was only done on samples with detected haemoglobin (Hb) variants on HPLC.

The cut-off level for haemoglobin A\textsubscript{2} of more than 4.0% was used for presumptive diagnosis of classical β-thalassaemia trait (George et al. 2001). But if it was more than 10%, a diagnosis of haemoglobin Lepore was considered, while the level more than 25% was interpreted as suggestive of haemoglobin E trait (Barbara 2006; Ryan et al. 2010). However, when the level was between 16 to 25%, haemoglobin E with alpha thalassaemia, co-inheritance was suggested (Barbara 2006; Ryan et al. 2010). The presence of other abnormal peaks in HPLC chromatogram was correlated with the demographic data and gel electrophoresis result.

All statistical analyses were conducted using the Statistical Package for Social Sciences for Windows SPSS 17.5 (SPSS inc., Chicago, IL, USA). The results were expressed as mean ± 2 standard deviation (SD).

**RESULTS**

Among 738 blood donors, 606 (82%) were males and 132 (18%) were females. The mean age was 33 years and SD was 10. The youngest donors were aged 17 years while the oldest were 61 years. Four hundred and thirteen (56%) were Malays, 162 (22%) were Indians, 148 (20%) were Chinese while 15 (2%) were others. The other races consisted of Iban 2, (0.3%), Kadazan 1 (0.2%), Kayan 1 (0.2%), Bugis 1 (0.2%) and Malaysian-Indonesian 1 (0.2%); while 9 (1.2%) donors did not specify their ethnicity. Seventy four (10%) of the blood donors were confirmed to have haemoglobin levels below 12.5 g/dL (8.7-12.4 g/dL), where four (5.4%) had haemoglobinopathies. Mean corpuscular haemoglobin (MCH) level lower than 27 pg was observed in 225 (30.5%) donors, of whom 35 (15.6%) displayed abnormal haemoglobin fractions on HPLC. Interestingly, haemoglobinopathy (haemoglobin A\textsubscript{2} prime) was found in one (0.1%) donor whose MCH value was within the normal range (28.2 pg) but the mean corpuscular volume (MCV) value was 79.7 fl. The other types of haemoglobinopathies detected with their haematological parameters shown in Table 1. All the abnormal cases were Malays except for the β-thalassaemia trait cases, three (60%) were Indians and two (40%) were Malays.

α-thalassaemia and normal haemoglobin A\textsubscript{2} β-thalassaemia carriers were not be able to be excluded in 190 (25.7%) cases based on our screening tests. The diagnosis would only be confirmed by molecular analysis. Male donors were predominant in this group 140, (73.7%). Malays accounted for 104 (54.7%), Indians 65 (34.2%) Chinese 20 (10.5%) and others one (0.5%). Forty six (24.2%) donors had haemoglobin less than 12.5 g/dL (8.7-12.4 g/dL) and the median haemoglobin level was 11.6 g/dL. The haematological parameters for these 190 undiagnosed cases shown in Table 2.
The severe types of thalassaemia that pose major public health problem in Malaysia are homozygous α⁰-thalassaemia (haemoglobin Bart’s hydrops foetalis) and β-thalassaemia major (Ainoon & Cheong 1994). Homozygous α⁰-thalassaemia is associated with perinatal mortality and potentially causing fatal pregnancy complications in mothers. Children with β-thalassaemia major are always manifested with severe anaemia after one year of age requiring lifelong blood transfusion. Since, they are transfusion dependent, iron chelators are important to overcome iron overload.

<table>
<thead>
<tr>
<th>Blood Indices</th>
<th>Hb E trait (n=19) 2.6%</th>
<th>Hb E/α (n=6) 0.8%</th>
<th>β-thalassaemia trait (n=5) 0.7%</th>
<th>Hb Constant Spring (n=2) 0.3%</th>
<th>Hb A2 prime (n=2) 0.3%</th>
<th>Hb Lepore (n=1) 0.1%</th>
<th>Alpha chain variant (n=1) 0.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>13.9 ± 1.3 (11.3-16.4)</td>
<td>14.1 ± 0.9 (13.0-15.1)</td>
<td>13.4 ± 1.2 (12.1-14.7)</td>
<td>13.7 ± 1.1 (12.9-14.4)</td>
<td>14.8 ± 0.1 (14.7-14.8)</td>
<td>14.0 ± 0.6 (13.4-15.6)</td>
<td>14.4 ± 15.5 (13.0-16.0)</td>
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<tr>
<td>Hct (%)</td>
<td>42.9 ± 3.4 (35.8-48.6)</td>
<td>44.6 ± 2.2 (40.8-47.1)</td>
<td>43.1 ± 3.5 (38.7-47.2)</td>
<td>44.0 ± 2.0 (42.6-45.4)</td>
<td>43.9 ± 2.3 (41.6-46.2)</td>
<td>46.7 ± 48.3 (41.0-51.0)</td>
<td>46.0 ± 50.1 (41.0-55.0)</td>
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<tr>
<td>RBC (10¹²/L)</td>
<td>5.7 ± 0.5 (4.8-6.8)</td>
<td>5.9 ± 0.4 (5.2-6.5)</td>
<td>6.6 ± 1.0 (5.0-7.9)</td>
<td>5.7 ± 0.3 (5.0-5.9)</td>
<td>5.6 ± 0.5 (5.2-6.0)</td>
<td>6.93 ± 5.82 (6.0-7.4)</td>
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<tr>
<td>MCV (fl)</td>
<td>75.1 ± 2.4 (71.3-79.3)</td>
<td>75.9 ± 4.8 (76.2-80.5)</td>
<td>66.3 ± 12.1 (58.4-87.3)</td>
<td>77.2 ± 7.4 (72.0-82.4)</td>
<td>78.6 ± 1.6 (77.4-79.7)</td>
<td>67.4 ± 83.0 (60.0-86.0)</td>
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<tr>
<td>MCH (pg)</td>
<td>24.3 ± 1.2 (22.4-26.8)</td>
<td>24.1 ± 2.1 (24.0-25.6)</td>
<td>20.7 ± 3.6 (18.3-26.7)</td>
<td>24.0 ± 3.0 (21.8-26.1)</td>
<td>26.5 ± 2.4 (24.8-28.2)</td>
<td>20.8 ± 26.6 (18.0-28.4)</td>
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<tr>
<td>MCHC (g/dL)</td>
<td>32.4 ± 1.1 (30.6-35.1)</td>
<td>31.7 ± 0.9 (30.0-32.3)</td>
<td>31.2 ± 0.5 (30.6-32.0)</td>
<td>31.0 ± 1.0 (30.3-31.7)</td>
<td>33.7 ± 2.3 (32.0-35.3)</td>
<td>30.8 ± 32.1 (29.0-34.0)</td>
<td>30.8 ± 32.1 (29.0-34.0)</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>38.4 ± 1.7 (34.0-42.0)</td>
<td>40.7 ± 3.5 (37.0-47.0)</td>
<td>37.3 ± 5.0 (34.0-46.0)</td>
<td>40.7 ± 0.7 (40.2-41.2)</td>
<td>43.0 ± 0.4 (42.7-43.2)</td>
<td>39.3 ± 39.9 (36.0-42.6)</td>
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<tr>
<td>Hb A₂ (%)</td>
<td>26.4 ± 0.8 (25.1-28.2)</td>
<td>23.5 ± 1.3 (21.0-24.4)</td>
<td>5.2 ± 0.9 (4.2-6.4)</td>
<td>2.6 ± 0.1 (2.5-2.7)</td>
<td>1.55 ± 0.1 (1.5-1.6)</td>
<td>11.7 ± 2.1 (10.0-13.1)</td>
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<tr>
<td>Hb F (%)</td>
<td>0.7 ± 0.3 (0.3-1.5)</td>
<td>0.7 ± 0.3 (0.3-1.2)</td>
<td>0.6 ± 0.4 (0.2-1.1)</td>
<td>1.2 ± 1.0 (0.5-1.9)</td>
<td>0.8 ± 0.6 (0.3-1.2)</td>
<td>3.1 ± 0.1 (1.0-3.2)</td>
<td>3.1 ± 0.1 (1.0-3.2)</td>
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</tbody>
</table>

Data reported as mean±SD

RBC: red blood cell
MCV: mean corpuscular volume
MCH: mean corpuscular haemoglobin
Hct: haematocrit
RDW: Red cell distribution width
MCHC: mean corpuscular hemoglobin concentration

Table 1: Haematological parameters of variant haemoglobin and β-thalassaemia trait

Table 2: Haematological parameters of undiagnosed cases

<table>
<thead>
<tr>
<th>Blood Indices</th>
<th>Mean ± SD</th>
<th>Value Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>13.6 ±1.5</td>
<td>8.7 - 16.9</td>
</tr>
<tr>
<td>Hct</td>
<td>43.5 ± 3.8</td>
<td>31.6 - 51.3</td>
</tr>
<tr>
<td>RBC (10¹²/L)</td>
<td>5.4 ± 0.5</td>
<td>4.0 - 6.8</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>80.1 ± 4.7</td>
<td>64.5 - 88.4</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.0 ± 1.8</td>
<td>17.7 - 26.9</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>31.2 ± 1.3</td>
<td>26.6 - 42.0</td>
</tr>
<tr>
<td>RDW (fl)</td>
<td>41.7 ± 4.0</td>
<td>12.0 - 51.0</td>
</tr>
<tr>
<td>Hb A2 (%)</td>
<td>2.6 ± 0.3</td>
<td>1.5 - 3.7</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>0.4 ± 0.5</td>
<td>0.1 - 5.0</td>
</tr>
</tbody>
</table>

DISCUSSION
complications. However, prior to year 2005, less than 20% of them receive adequate treatment (George 2001). This not only affects the clinical health status, but also gives a great impact on our socio-economy. Hence, establishment of screening programme for identification of thalassaemia carrier is extremely crucial. The most effective approach is the implementation of screening programme among school students, premarital couples, family members of thalassaemia patients and pregnant women during antenatal booking (Bianco et al. 1984; Samavat & Modell 2004; AlHamdan et al. 2007).

But a feasible option is to also test blood donors as the blood sample is readily available at time of blood donation. In general, this approach can enhance our national screening programme since the response rate is poor in our community (Anjanna et al. 2011; Wong et al. 2011). Lack of knowledge and unwillingness for screening were the main reason for the poor response (Anjanna et al. 2011; Wong et al. 2011).

Few studies reported the success of thalassaemia screening programme at school but few have raised arguments against this practice (Troitskaia et al. 1999; AlArrayed et al. 2003; Li et al. 2009). School students are considered legally minors. Their assents in participating carrier testing may be influenced by friends, teachers and guardians (Ross 2006). The possibilities of confusion between carrier state and being affected by the disease might occur in the absence of proper counselling and this can lead to adverse psychological reaction in those with positive results (Axworthy et al. 1996; Marteau et al. 1992; Stewart-Brown & Farmer 1997). Based on ethical issues, genetic testing like thalassaemia screening can only be carried out if immediate diagnosis is necessary, for instance when an adolescent is pregnant or planning for pregnancy (ASHG/ACMG 1995). At this age, screening would be acceptable. The test should be deferred until they are able to justify the appropriate time to have the screening (Frumkin & Zlotogora 2008). Adolescents aged 18 and above are generally legal for consent because (ASHG/ACMG 1995) they are considered mature and capable in making judgment for their future. Married blood donors who are identified as thalassaemia trait would alert their spouses to get a similar test in order to ascertain their carrier status. If they are at risk of having children with thalassaemia major, they would then be counselled to undergo prenatal diagnostic test, so that they can have the option of either continuing or terminating the affected pregnancy. This, however results in difficult decision making caused by misapprehension of the disorders, cultural values and religious belief (El-Hazmi 2009; Wong et al. 2011). Premarital screening would be an alternative way since this approach might be more effective in the Malay community. It may identify carrier couples, thus giving them the opportunity to circumvent affected pregnancies. Studies showed that the incidence of infants born with the disease reduced tremendously by implementation of premarital screening (Zlotogora 2009).

In the present study we found that 36 (5%) of 738 healthy blood donors
were presumptively diagnosed as thalassaemia carriers. The frequency of haemoglobin E carrier was similar with the previous study (George & Khuziah 1984), but the distribution of β-thalassaemia carrier and heterozygous haemoglobin Constant Spring were below the reported incidence (George 2001; Wee et al. 2009). α-thalassaemia and normal haemoglobin A2 β-thalassaemia could not be excluded in 190 (25.7%) blood donors in this study because no abnormal pattern was noted in HPLC result. Further confirmation by DNA analysis on these cases demand for futher analysis in the future.

Anaemic blood donors are no longer an issue, because further workup will be initiated to find out the cause of anaemia. Nonetheless, for those who pass copper sulphate test, they are always considered to have normal haemoglobin level. Therefore they will be permitted for blood donation and missed from detection of thalassaemia trait. A few studies had found this method was less sensitive in detecting anaemia compared with other portable haemoglobinometry devices available in the market (Newman 1997; Nadarajan & Eow 2002; Boulton 2008). It is not cost-effective to include haemoglobin analysis as routine blood test in blood donors in order to screen for thalassaemia carrier. Besides, it is troublesome to potential blood donors and can negatively impact the blood donor return. In this study, majority (70%) of the blood donors had results within normal limits. A local study which included anaemic blood donors also found that 84% of them had normal haemoglobin electrophoresis pattern (Rosline et al. 2006). Therefore, performing haemogram on initial diverted blood from collateral pouch after blood donation would be more worthwhile. More accurate haemoglobin level would be measured and more detail blood cell indices would be scrutinized. Based on these results, certain donors will be selected for complete haemoglobin analysis.

According to British Committee for Standards in Haematology (British Journal of Haematology 2010), MCH value is the recommended parameter for thalassaemia screening as it is more stable than MCV. We had only one case with detected haemoglobin variant on HPLC, yet the MCH value was normal (more than 27 pg) while the MCV value was 79.7 fL. A previous study that adopted MCV level of less than 80 fL as a criterion for further analysis in apheresis blood donors had found that 36% of the donors had haemoglobinopathy (Bryant et al. 2009). Such findings showed that both MCV and MCH values are essential for prediction of thalassaemia trait.

It is highly debated whether or not thalassaemia and haemoglobinopathy screening should be done for regular blood donors to avoid transfusion of dysfunctional red cells and transfusion acquired haemoglobinopathy. Rational for screening most often depends on the demographic frequency of thalassaemia and clinically significant haemoglobinopathies in a given population and special requirement of recipients. In addition to this, post transfusion lifespan of erythrocyte is also an important factor in transfusion
Thalassaemia Screening in Healthy Blood Donors

CONCLUSION

Thalassaemia screening among healthy blood donors could benefit the existing thalassaemia screening programme in Malaysia. It is a feasible and effective approach in detecting thalassaemia carriers in our population. However, a more cost-effective screening method is suggested since prevalence of thalassaemia carrier in healthy blood donors are low. Haemogram is an appropriate screening test as scrutinisation of blood indices may allow selection of blood donors for further analysis of haemoglobin.

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REFERENCES


