Screening for thalassaemia among group of students of a higher institution - our experience
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ABSTRACT
Introduction: Thalassaemia is an inherited blood disorder and is a significant public health alarm in Malaysia with many not knowing they are carriers of this haemoglobin disorders. Materials and methods: This study conducted a one off collection of blood samples from 72 Malays students of International Islamic University Malaysia (IIUM) in Kuantan. Blood samples were subjected to conventional haemoglobin analyses that include full blood count and picture, HPLC, Haemoglobin electrophoresis and H-inclusion test. All samples were also genotyped for alpha thalassaemia-1 of Southeast Asia (α-Thal1SEA). Result: There were 17(23.6%) students who were diagnosed as thalassaemia carriers. Out of this, four (5.5 %) and six (8.3 %) students were presumptive β-thalassaemia trait and Haemoglobin-E trait as determined by the HPLC assay respectively. Nine (12.5%) students were genotyped α-Thal1SEA among whom two were also β-thalassaemia carriers. All thalassaemia cases had MCH of < 27pg. Nonetheless, two out of six Haemoglobin-E trait and three out of nine α-Thal1SEA carrier had MCV value of >80fL. Two out of four (50%) presumptive β-thalassaemia trait and one out of six (17%) students of presumptive Haemoglobin-E trait had family history of thalassaemia respectively. Conclusion: The high occurrence of the three common types of thalassaemia carrier (B, Hb-E and α-Thal1SEA thalassaemia) in our small group of subjects could be due to better participation of students who had family history of thalassaemia. The study reaffirmed the importance of molecular study for detection of alphathalassaemia and the use of MCH value of <27pg rather than MCV value of < 80fL for prediction of thalassaemia.

KEYWORDS: Thalassaemia, screening, diagnosis, genetic carriers.

INTRODUCTION
Thalassaemia is a group of inherited autosomal recessive blood disorders characterized by impaired or the absence of production of normal α or β globin chain peptide. These groups of single gene disorders have an estimated 5% prevalence as carriers. In Malaysia the prevalence is estimated to range between 3% and 50% for Haemoglobin-E (Hb-E), 3% and 16% for α-thalassaemia and slightly lower for β-thalassaemia and Constant Spring carriers of 3% and 4% and 1% and 4% respectively. The estimated prevalence for Hb-E and alpha thalassaemia in Malaysia is similar to the reported prevalence in Thailand, a neighboring country.

Symptomatic thalassaemia major and intermedia requires life long-term medical care and support. Population screening for carrier detection has been advocated to reduce it occurrence and is being practiced in many countries. The best mechanism to do this, however, is still debatable since this would require huge financial resources to cover various areas such as the counseling service and genetic support. In Malaysia, most community health centers are equipped with blood cell analyzer, and it is therefore feasible to adopt the screening method based on the MCV of <80fL or MCH of <27 pg.

In this study, a complete work-up for thalassaemia which include conventional methods for haemoglobin analyses and molecular study for α-Thal1SEA deletion were performed in our group of students who responded to our advertisement for thalassaemia screening. To mimic on site population screening, a one off blood collection was done.

MATERIALS AND METHODS
Subjects and blood analyses
The entire study was carried out over a period of three months following approval from the ethics and research committees of International Islamic University of Malaysia (IIUM). Study subjects were medical and science students of the IIUM, who were recruited by announcement on notice boards. To mimic a population-screening programme, blood sample from all 72 Malay students aged 20-25 years was collected on the same day.

The handling and storage of blood samples were in accordance with standard laboratory practice. HPLC
The chromatographic profiles and the HbA2 and Hb F levels determined by HPLC assays were interpreted based on the study by Fucharoen et al.⁷ Thus, a presumptive β-thalassaemia trait was made when HbA2 level was from 4.2 to 6.8% while presumptive Hb-E trait and Hb-E homozygous were made when the HbA2 was from 25% to 36% and 85% to 95% respectively. All samples were subjected to α-Thal1SEA molecular study regardless of the HPLC finding. Results were analysed using the Statistical Package for Social Sciences version 11 (SPSS Inc., Chicago, Illinois, USA). A p value of < 0.05 was considered as statistically significant.

Molecular analysis of α-Thal1SEA thalassaemia. DNA was extracted by standard method using column separation method (QiaAmp Blood Mini Kit, Qiagen). The three primers for α-Thal1SEA genotyping were as described by Chang et al.⁸ The Gap Polymerase Chain Reaction assay consisted of 200 µm of each dNTP, 2.5 mM MgCl₂, 1X Q solution (Qiagen, Hilda, Germany), 2.5 U HotStar Taq DNA polymerase, 100-200 ng of genomic DNA and distilled water made to 50 µL of cycle reaction. Reactions were run in thermal cycler (9700 GeneAmp, Applied Biosystem, USA). An initial 10 minutes denaturation at 96°C was followed by 35 cycles of 96°C denaturation, 60°C annealing and 72°C extension, each for 30 seconds. A final extension at 72°C for 10 minutes ended up the reaction. All amplified products were run on 1.5% agarose gel electrophoresis and viewed on gel documentation system. The expected product for heterozygous α-Thal1SEA deletion is 314 and 195 bp whilst the homozygous α-Thal1SEA deletion is 195bp. The expected product for α-Thal1SEA negative amplicon is 314bp. The DNA of a known α-Thal1SEA thalassaemia patient was used as the positive control. Positive samples were repeated at least once.

RESULTS
Of the 72 Malay students who responded for thalassaemia screening, the majority was females (87.5%). The haemoglobin (Hb) levels of all the students were normally distributed with the mean Hb of 13.9 g/dL in males; and 12.3 g/dL in females. Ten students (13.9%) had low Hb level of whom only one was a male student (cut off Hb< 11.5 g/dL for female and < 12.5 g/dL for male).

There were four (5.5%) students with a presumptive β-thalassaemia trait and six (8.3%) with Hb-E trait. Two out of four students with the presumptive β-thalassaemia trait was a twin who had strong family history of clinical thalassaemia. The molecular analysis detected nine (12.5%) students with α-Thal1SEA genotype (Figure 1), two of whom were also β-thalassaemia carriers detected earlier through HPLC assay. None had a positive H inclusion test. In total of 17 (23.6%) students were diagnosed as thalassaemia carriers (presumptive or through molecular study).

<table>
<thead>
<tr>
<th>Table 1: Data on MCV values and types of thalassaemia</th>
<th>MCV ≥ 80 fl</th>
<th>MCV &lt; 80 fl</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-thalassaemia trait</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Non B-thalassaemia trait</td>
<td>53</td>
<td>15</td>
<td>0.004</td>
</tr>
<tr>
<td>Hb-E trait</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Non Hb-E trait</td>
<td>51</td>
<td>15</td>
<td>0.038</td>
</tr>
<tr>
<td>--sea/αα</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Non --sea/αα</td>
<td>50</td>
<td>13</td>
<td>0.008</td>
</tr>
</tbody>
</table>

*Fisher-exact test
Out of 72 samples studied, 19 (26.38%) subjects had both low MCV and MCH value. All subjects diagnosed as thalassaemia carrier had MCH of <27 pg thus further statistical analyses on this parameter were deemed unnecessary. All the presumptive β-thalassaemia trait subjects had MCV levels of <75 fL. There was a significant association between the MCV level of <80 fL with α-Thal1SEA thalassaemia (two tails Fisher’s Exact test, p=0.008) and MCV level of <80 fL with Haemoglobin-E trait (Fisher exact test, p=0.038) (Table 1). Nevertheless, the MCV value was normal (>80 fL) in two out of six subjects with presumptive diagnosis of Hb-E trait and three out of nine subjects with α-Thal1SEA. The median of Hb irrespective of sex for β-thalassaemia carriers was 9.8 g/dL, while the mean for Hb-E trait and α-Thal1SEA carriers were 13.3 g/dL and 11.5 g/dL respectively. The median Hb for subjects with concomitant evidence of β-thalassaemia and α-Thal1SEA was normal at 12.5 g/dL. There were two subjects with hypochromic microcytic anemia but with normal haemoglobin analysis findings. No further investigation was done to determine another possible cause/s of anemia.

Counseling was done for students with presumptive β-thalassaemia carriers and Hb-E carriers but none of α-thalassaemia carrier since the result was only available after the students left the university. Two out of four students of presumptive β-thalassaemia carriers and one out of six students of Hb-E carriers had family members with thalassaemia. Family history of thalassaemia could not be determined in all the cases of SEA thalassaemia carriers except in one who was also positive for β-thalassaemia carriers and had family history of thalassaemia.

**DISCUSSION**

The standard protocol for screening of thalassaemia normally follows a defined flowchart wherein the erythrocyte indices are taken as the preliminary test. Generally only those with MCV level of <80 fL and/or MCH level of <27 pg are further tested using HPLC and molecular analyses. In Malaysia, MCH level of <27 pg is adopted as the cut-off value. The MCV level was of special interest in our study subjects since blood samples were collected and analysed within 24 hours of collection and has been the preferred indices in other screening centres.9

Even though the MCV value of < 80fL was used for definition of microcytic red blood cells, all the presumptive β-thalassaemia trait subjects in our study had MCV level of <75 fL, which was similar to previous observations.10 Mah et al11 however, found that using a cut of value of <75 fL had led to missing detection of almost 3% of β-thalassaemia carriers and 4% of α-Thal1SEA carrier subjects in the Chinese population, while the use of standard cut-off value of 80 fL could detect both carrier states. The second observation was not replicated in our study as three out of nine (33.3%) of α-Thal1SEA genotyped subjects had MCV of > 80 fL. Our data agrees with the findings of previous studies that concluded a proportion of Hb-E and α-thalassaemia carrier individuals would be missed if the MCV <80 fL cut-off value was used.11,12 Using the MCH value is most probably the preferred choice because in practice it might be difficult to analyze MCV values within six hours of collection. This is especially true if the collection centres are at the remote areas. In our study, we found all the thalassaemia carrier subjects had MCH value of less than <27 pg. This approach, however, is not without deficiency as some Hb-E trait and α-thalassaemia subjects in a larger study were shown to have higher MCH value.13 All of our HbE trait subjects (n=6) had low MCH whilst two subjects had MCV>80fL. Thus, MCH rather than MCV was the preferred parameter for screening of thalassaemia. Using the preset cut-off MCV value alone is confounded with missed detection of Hb-E trait.

The use of H-inclusion test for screening of α-thalassaemia is an observer dependent, laborious and has a poor sensitivity. Even though the staining procedures were carried out by following the standard H-inclusion protocol, fresh sample of control positive blood was not available on the day of this examination. This could contribute to the failure in identifying any positive H-inclusion in all subjects with α-Thal1SEA genotype. We believe the observer lacked of experience in interpreting H-inclusion test had also contributed to this failure.

The finding of this study does not reflect the prevalence of thalassaemia among Malays in Malaysia since the sample size was very small. However, it was observed that the distribution of Hb-E carriers among our Malay students were within the range of the previous review on thalassaemia in the general population, and not very different either to a recent study done in Thailand.14 There were over representation of α-Thal1SEA and β-thalassaemia carrier subjects as compared to previous studies.15,16 It should be noted, however, that data from our study was very similar to the prevalence observed amongst Malays in Singapore,17 a southern neighbouring country that has similar multiracial communities. The percentage of Hb-E and β-thalassaemia carriers were also quite similar to the data done on blood donors.18 The study, however, did not examine for α-Thal1SEA genotype. As this purpose of study was not to determine the prevalence of thalassaemia in general community, the high occurrence of thalassaemia in our subjects could be due to significant participation from students who had a family history of thalassaemia. The over-representation of subjects with α-Thal1SEA genotype could also be due to the contribution from subjects of mixed ethnic groups but were identified as Malay in this study.

**CONCLUSION**

This study reaffirms the importance of molecular study for detection of alphaβ-thalassaemia carrier. The MCH value of < 27pg is the preferred parameter for prediction of thalassaemia carrier as the use of MCV of < 80fL has led to miss detection of some Haemoglobin-E and α-Thal1SEA carrier. Thalassaemia is prevalent in Malays; this study supports the opinion that popula-
dition screening for common types of thalassaemia is recommended.

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REFERENCES