SHORT COMMUNICATION

Thalassaemia Screening Among Students in A Secondary School in Ampang, Malaysia

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SUMMARY

Thalassaemia is a common disorder in Malaysia. It is estimated that 4.5% of the population are carriers for β- or α- thalassaemias. We set out to screen Form 4 students aged between 15 and 16 years old in a national school, for thalassaemia in March 2008. Written consent was obtained from 310 students. The carrier rate for the common thalassaemia syndromes was 6.8% (2.9% for β-thalassaemia, 2.6% for HbE and 1.3% for two-gene deletion for α-thalassaemia). Carriers for β-thalassaemia and two-gene deletion for α-thalassaemia were more common in the Chinese (4.3% and 1.4% respectively) while heterozygous HbE was more common in the Malays (3.8%). The laboratory cost of screening one student was RM 45 and the total number of man-hours spent in this screening activity was 600. This screening exercise showed that thalassaemia carriers are common among the Chinese and Malays and it is feasible to carry out a screening programme for secondary school students.

KEY WORDS:
Thalassaemia, β- thalassaemia, α- thalassaemia, Screening, Carrier

INTRODUCTION

Thalassaemia is an autosomal recessive disorder and the thalassaemic syndromes are inherited from both parents who are carriers for the disorder. It is a major health problem in Malaysia. It was estimated that there were 2500 transfusion-dependent thalassaemics in 1999 throughout the country based on hospital patient lists and 3500 in 2008 [1]. The common thalassaemia syndromes in Malaysia are β-thalassaemia major, E-β-thalassaemia, Hb H disease and Hb Barts hydrops fetalis [2]. About 4.5% of people are carriers for β-thalassaemia and couples who are carriers are at risk of having a child with β-thalassaemia major. Patients with β-thalassaemia major require life-long blood transfusions and iron chelation to prevent complications from iron overload. About 4.5% are also carriers for α-thal 1 or α^0 thalassaemia (2 α-gene deletion), which is more common in the Chinese than Malays. Couples who are carriers for α-thal 1 are at risk of having a fetus with Hb Bart's hydrops fetalis [3]. Treatment remains complex, burdensome and expensive. Prenatal diagnosis is still not widely available in Malaysia and selective abortion of affected fetuses is not widely accepted. Our objective of this study was to determine the thalassaemia carrier rate among students in a secondary school and to to educate identified healthy carriers the risk and options for preventing the birth of children with severe thalassaemia syndromes.

MATERIALS AND METHOD

Consent forms for screening were sent out to all Form 4 students and their parents one week prior to screening. Blood samples were taken for full blood count, haemoglobin analysis and iron studies over 4 separate days. All samples were subjected to full blood count using Sysmex XE 5000 (Kobe, Japan) and haemoglobin analysis by capillary electrophoresis[4] using Sebia Capillarys 2 Version 5.50 (Cedex, France). All hypochromic (mean cell haemoglobin, MCH< 27 pg) and microcytic (mean cell volume, MCV< 80 fl) samples with normal HbA2 (≤3.5%) were subjected to measurement of serum iron and total iron binding capacity (TIBC) as well as DNA analysis for alpha thalassaemia mutations by the multiplex polymerase chain reaction and with allele-specific amplification using an amplification refractory mutation system (ARMS) methods. The α-thalassaemia mutations that were screened for were the deletional forms by multiplex PCR method [5] were single gene deletion $-\alpha^{3.7}$ and $-\alpha^{4.2}$; and two-gene deletions $--^{SEA}$, $--^{FIL}$, $--^{MED}$, $--(\alpha)^{20.5}$ and $--^{THAI\ (3)}$ and the non-deletional forms by multiplexed ARMS method [6] which included initiation codon (ATG→A-G), codon 30 (ΔGAG), codon 35 (TCC→CCC), codon 59 (GGC→GAC), codon 125 (CTG→CCG) or Hb Quong Sze and termination codon (TAA→CAA) or Hb Constant Spring).

RESULTS

a. Consent

Three hundred and ten students (74.3%) out of a total of 417 students consented to be screened for thalassaemia. All were aged between 15-16 years old (Table I). More than 90% had some knowledge about thalassaemia. All identified carriers were counselled about thalassaemia.

b. Hb analysis & Hypochromic microcytic indices

All samples were subjected to Hb analysis (Table II). Hypochromasia (MCH< 27 pg) and microcytosis (MCV< 80 fl)

This article was accepted: 20 September 2011
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Table I: Sex distribution and ethnic groups of students screened

Ethnic	Male	Female	Total (%)
Chinese	75	62	137 (44.2)
Malay	46	84	130 (42.0)
Indian	18	21	39 (12.5)
Sikh	1	0	1 (0.3)
Iban	1	2	3 (0.96)
Total	141	169	310 (100)

Table II: Full blood count and Hb analysis results for the detected carriers

Type of Hemoglobinopathies (N; Sex)	Hb (g/dL) Mean (range)	MCV (fl) Mean (range)	MCV (fl) Mean (range)	Hb A2 (%)	HbE/ J/H/D (%)
β-trait (N=9)	13.8	60.9	19.9	5.6	
M=6	(10.8-14.5)	(55.6-73.3)	(17.6-23.8)	(5.2-6.3)	-
	11.4	62.3	20.1	5.6	
F=3	(11.—12.5)	(57.1-69.0)	(18.0-22.1)	(4.9-5.7)	-
HbE trait (N=7)	14.0	73.1	24.4	3.5	24.9
M=2	(14.0-14.1)	(70.3-76.0)	(23.2-25.6)	(3.5-3.6)	(24.5-25.3)
	12.7	71.9	24	3.3	26.6
F=5	(12.3-13.0)	(71.2-76.3)	(22.8-25.0)	(3.1-3.7)	(24.8-27.9)
Hb J (N=1; M)	14.5	90	29.7	2.6	51.3
HbH disease (N=1; F)	9.0	55.8	17	1.3	1.3
HbD (N=1; M)	14.5	69.5	23.3	3.4	34
Total of patients: 19					

Notes: F=Female, M=Male, N=number of person

Table III: Detected alpha thalassaemia mutations, red cell indices and iron studies

α-thalassaemia mutations		Hb (g/dL) Mean (range)	MCV (fl) Mean (range)	MCH (pg) Mean (range)	Ser Iron (μmol/L)	TIBC (μmol/L)
het -$\alpha^{3.7}$	4	13.5	77.0	25.0	10.3	64.0
		(12.2-15.0)	(76.4-78.7)	(24.5-25.3)	(7.8-15.1)	
het --^{SEA}	3	13.8	62.6	20.4	21.7	64.3
		(12.6-14.0)	(61.0-63.8)	(19.1-20.5)	(10.7-27.7)	(54.6-72.0)
het --^{FIL}	1	11.5	63.0	20.0	4.7	48.0
het -$\alpha^{4.2}$	1	14.3	74.0	24.8	19.2	67.8
Hb Quong Sze (CTG-CCG)	1	11.8	75.0	23.4	7.7	54.1
Total of patients: 10						

Notes: N=number of person

were identified in 56 samples (18%). Out of these, 20 were iron deficient (serum iron< 7 μmol/L and TIBC> 70 μmol/L) with anaemia in 3 students (all were females with Hb<11.0 g/dL). No cause was identified for 8 hypochromic microcytic samples. All students heterozygous for HbE had hypochromic microcytic indices.

c. Alpha thalassaemia mutations

Alpha thalassaemia mutations were identified in 10 students with hypochromic microcytic indices and normal HbA2 (α-thalassaemia mutation analysis was not carried out for heterozygous HbE and HbD). Heterozygous two-gene deletions had MCV below 70.0 fl and MCH below 22.0 pg. (Table III).

d. Carrier and ethnic distribution

The carrier rate for all thalassaemias was 9.3%. The carrier rate for β-thalassaemia, HbE and two-gene deletion for α-thalassaemia was 2.9%, 2.6% and 1.3% respectively. An observation from this research with data available from small sample size of this study showed the heterozygous β-thalassaemia was higher in the Chinese as compared with Malays (4.3% vs 1.5%) while heterozygous HbE was frequently detected in the Malays (3.8%vs 1.4 %). The observed difference was not able to indicate s statistical significant of this distribution due to sample small size. Two-gene deletion for α-thalassaemia was seen in 1.4% of Chinese and 0.7% in Malays. One Malay student was diagnosed with HbH disease.

DISCUSSIONS

We demonstrate that the carrier rate for the common thalassaemia in Malaysia was approximately 7%. This study also revealed the high frequency and the high acceptance rate among this secondary school student which makes populations screening on wider scale feasible [4].

This study also identified a significant rate of iron deficiency, a treatable condition in 6.4% in asymptomatic adolescents in a highly urbanised setting. It may be postulated that the 8 students with hypochromic microcytic red cell indices of indeterminate cause might also have had iron deficiency which could not be identified due to suboptimal sensitivity of the diagnosis of iron deficiency using serum iron and TIBC measurements.

The screening expenditure for this project was RM 27,739.76 included 34 patients subjected for DNA analysis. The cost of laboratory tests to screen one student was RM 74.06. Although these are rough estimates which are not necessarily applicable in a more inclusive national level screening, the figures contrast favourably with the cost of treating a patient with transfusion-dependent thalassaemia. Transfusion-dependent thalassaemics require an intensive transfusion programme coupled with iron chelation to prevent complications from iron overload. Present estimates suggest that local cost of one unit of packed red cells is RM 200.00 (National Blood Centre, Malaysia). The life-time cost in treating one patient with β-thalassaemia major is estimated to be £219,608 (RM 1,000,000)[5]. The experience of other countries with a high health burden due to thalassaemia suggests that the birth rates of patients with β-thalassaemia major may be significantly reduced by widespread screening and education programmes. In Cyprus, the number of births of children with β-thalassaemia major declined by 75% in 1988 with these preventive strategies[6].

CONCLUSION

It is important for Malaysia to embark on a thalassaemia screening and education programme to identify carriers and to counsel them so that thalassaemia will no longer be a health burden to the country but a preventable disease.

ACKNOWLEDGEMENT

We would like to thank the Director General of the Ministry of Health for the permission to publish this paper, the Director of Hospital Ampang and staff of the Haematology Department, Hospital Ampang, for supporting this work. Our appreciation is extended to Dr Salwana Mohd Ali, Sister Zuraidah Amir and the thalassaemia nurses of Ampang Hospital in helping with the screening and counselling.

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