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Screening for hemoglobinopathies among patients in a government hospital and health clinics in Perlis, Malaysia

Chin Yuet Meng*, Ezalia Esa, Aliza Mohd Yacob, Saritha Ramachandran, Zubaidah Zakaria
Hematology Unit, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract

The hemoglobinopathies include all genetic diseases of hemoglobin (Hb) and fall into two main groups: the thalassemias and structural hemoglobin variants (abnormal hemoglobins). Thalassemia is a public health problem in Malaysia. About 4.5% of the Malays and Chinese are β-thalassemia carriers. We performed hemoglobin analysis on a total of 499 patients from a Government Hospital and Health Clinics in the state of Perlis, Malaysia. About 91.4% of the patients were Malays. All patients had microcytic hypochromic anemia except for a few who went for thalassemia screening. Female patients outnumbered male patients in the ratio of 3.5:1. About 75.7% of the female patients were of childbearing age (17 - 40 years) and a majority of them were there for their antenatal checkup. Using our screen tests (full blood count, high performance liquid chromatography (HPLC), and agarose gel electrophoresis), the common hemoglobinopathies detected were HbE trait (19.3%), β-thalassemia trait (14.6%), HbH disease (1.8%), Hb Constant Spring (1.6%), Homozygous HbE (1.4%), and HbE- β-thalassemia (1.4%). Thalassemia is preventable through screening and education programmes, and prenatal diagnosis. Thalassemia screening is provided free of charge at various government hospitals and health clinics throughout the country.

Key words: Hemoglobinopathies screening, β-thalassemia trait, HbE trait, Thalassemic diseases

*Corresponding Author: Dr Chin Yuet Meng, Hematology unit, Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur, Malaysia. E-mail: chinym@imr.gov.my

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Introduction

Hemoglobinopthies are the most common inherited monogenic disease in the world. The hemoglobinopathies include all genetic diseases of hemoglobin (Hb) and are autosomal recessive. Hemoglobinopathies fall into two main groups: the thalassemias and structural hemoglobin variants (abnormal hemoglobins). The thalassemias are characterized by the absence or reduction in the synthesis of one or more of the globin chains of hemoglobin, and the structural variants are due to substitution of one or more amino acids in the globin chains. The two main types of thalassemia are the alpha (α) and beta (β) thalassemias; and the common structural Hb variants are HbS, HbE and HbC. The different combinations of the subtypes result in the heterogeneous clinical nature of the hemoglobinopathies, ranging from mild hypochromic anemia to severe, lifelong, transfusion dependent anemia with multiorgan involvement [1].

Alpha and β-thalassemia, HbE and Hb Constant Spring (CS) are prevalent in Southeast Asia (SEA). These abnormal genes in different combinations give rise to more than 60 different thalassemia syndromes. The four main thalassemic diseases in SEA are Hb Bart's hydrops fetalis (homozygous α-thalassemia), homozygous β-thalassemia, HbE β-thalassemia and HbH disease [2]. Hb Bart's hydrops fetalis results in death in utero and maternal complications. Homozygous β-thalassemia (β-thalassemia major) causes severe anemia, requires transfusion throughout life, and daily iron chelation therapy for survival.

The 2010 Population and Housing census of Malaysia (Census 2010) [3] showed that Malaysia is

a multi-ethnic country with a population of 28.3 million consisting of Malays and other Bumiputera groups (67.4%), Chinese (24.6%), Indians (7.3%) and other ethnic groups (0.7%). About 4.5% of the Malays and Chinese are β-thalassemia carriers [4]. Thalassemia is a public health problem in Malaysia. All thalassemia major patients have access to iron chelating treatment. The National Thalassemia Registry as of May 2010 recorded a total of 4,768 transfusion-dependent thalassemia major patients, with each patient requiring about US $294 – $882 for iron chelation therapy per month [5]. These expensive treatments are an economic burden to the country. Hb analysis for detection of hemoglobinopathies is performed as a routine diagnostic test in our Hemoglobinopathy Laboratory. The objective of this study was to screen for hemoglobinopathies among patients in a Government Hospital and Health Clinics in the state of Perlis, Malaysia.

Materials and Methods

A total of 499 patients from a Government Hospital and Health Clinics in Perlis were included in this retrospective study. There were 112 male (22.4%) and 387 female patients (77.6%). Out of 499 patients, 456 patients (91.4%) were Malays, 15 (3.0%) were Chinese, 3 (0.6%) were Indian, and 25 (5.0%) were of other ethnic groups. Most of the patients in the other ethnic groups were Siamese as Perlis has the Provinces of Thailand at its northern border. The age of patients range from 1 to 75 years. Full blood picture (FBP) performed at the Hospital and Clinics showed microcytic hypochromic anemia in all the patients except for a few of them who went for thalassemia screening. About 2.5 ml of blood was collected in a tube containing EDTA from each patient and the blood samples were sent to our Hemoglobinopathy Laboratory, Hematology Unit, Institute for Medical Research, Kuala Lumpur for Hb analysis. Patients less than one year of age were excluded from the study.

All blood samples were subjected to full blood count (FBC) using Cell Dyn 1800 (Abbott Laboratories, USA), and Hb analysis by high performance liquid chromatography (HPLC) using Bio-Rad Variant II Hemoglobin Testing system (Hercules, USA). Agarose gel electrophoresis (AGE) using Sebia Hydrasys (Cedex, France) at alkaline pH was performed on all cases with mean cell volume (MCV) < 80 fl , mean cell hemoglobin (MCH) < 27 pg, or if an abnormal Hb was detected on HPLC. Sickling test and gel electrophoresis at acid pH was performed if necessary. Using HPLC, the normal values for HbA2 are 2.2 – 3.4%, and for HbF <2%. HbA2 cut-off levels of between 4.0 - 8.0% using HPLC was identified as β - thalassemia trait [6, 7]. All data were analysed using IBM SPSS software version 21.

Results

a) Reasons for going to Hospital and Health Clinics

The reasons why these patients go to the Hospital and Health Clinics were as follows: Anemia (49.1%), thalassemia screening (41.9%), and other medical reasons (9.0%). About 293 out of 387 (75.7%) female patients were of child bearing age of between 17-40 years, and a majority of them were at the health clinics and hospital for their antenatal checkup (ANC). The reasons patients went for thalassemia screening were children, spouse, siblings, parents or relatives had thalassemia. Some patients voluntarily requested for thalassemia screening to rule out hemoglobinopathy.

b) Types of Hemoglobinopathies

The Hb analysis results of the 499 patients are shown in Table 1. Out of 499 patients, 42 patients (8.4%) were within normal limits using our screen tests, 244 patients (48.9%) were diagnosed as having normal Hb subtypes but does not rule out α -thalassemia and normal Hb A2 β-thalassemia (DNRO), 96 patients (19.3%) had HbE trait, 73 patients (14.6%) had β-thalassemia trait, 9 patients (1.8%) had HbH disease, 7 patients (1.4%) had HbE β-thalassemia, 8 patients (1.6%) had Hb Constant Spring (HbCS), 7 patients (1.4%) had homozygous HbE, and 13 patients (2.6%) had other types of hemoglobinopathies. The other types of hemoglobinopathies include borderline β-thalassemia trait (3 patients), β-thalassemia intermedia (2 patients), HbH disease with CS (4 patients), HbS trait (2 patients), Hb variant (1 patient), and deta-beta thalassemia (1 patient). The Hb analysis results of the 456 Malay patients (Table 1) are as follows; Within normal limits: 9.0%, DNRO: 47.8%, HbE trait: 20.0%, β-thalassemia trait: 14.5%, HbH disease: 1.5%, HbCS:1.5%, HbE β-thalassemia: 1.5%, homozygous HbE: 1.5%, and other types of hemoglobinopathies: 2.7%. There were one within normal limits, 8 DNRO, 4 β-thalassemia trait, one HbCS, and one borderline β-thalassemia trait among the 15 Chinese patients. The 3 Indian

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patients were diagnosed as DNRO. In the other ethnic groups (majority are Siamese) there were 15 DNRO, 3 β-thalassemia trait, 5 HbE trait and 2 HbH disease.

Table 1: Types of hemoglobinopathies among patients in a Government Hospital and Health Clinics in Perlis

ETHNIC GROUP	Type of Hemoglobinopathies									TOTAL (*n*)
	N	DNRO	E trait	β- trait	HbH	HbCS	Homo E	E- β thal	Others	
Malay	41 *(9.0%)*	218 *(47.8%)*	91 *(20.0%)*	66 *(14.5%)*	7 *(1.5%)*	7 *(1.5%)*	7 *(1.5%)*	7 *(1.5%)*	12 *(2.7%)*	456 *(100%)*
Chinese	1	8	0	4	0	1	0	0	1	15
Indian	0	3	0	0	0	0	0	0	0	3
Others	0	15	5	3	2	0	0	0	0	25
TOTAL (*n*)	**42 (8.4%)**	**244 (48.9%)**	**96 (19.3%)**	**73 (14.6%)**	**9 (1.8%)**	**8 (1.6%)**	**7 (1.4%)**	**7 (1.4%)**	**13 (2.6%)**	**499 (100%)**

β trait : β-thalassemia trait
DNRO: Normal Hb subtypes but does not rule out α-thalassemia & normal HbA2 β-thalassemia
E-β thal: HbE β-thalassemia
n: Number of Patients
HbCS: Hb Constant Spring
HbH: HbH disease
Homo E: Homozygous HbE
N: Within normal limits
E trait: HbE trait

Table 2: Reasons for going to Government Hospital and Health Clinics, and age at diagnosis of HbE β- thalassemia patients

Patient No.	Age (years)	Sex	*Reasons
1A	70	M	Anemia
2A	28	F	Anemia
2A-S	1	M	Thal screening
3A	32	F	NIDDM, HPT
4A	1	F	Anemia
5A	19	M	Fever, jaundice
6A	22	F	Thal Screening

Table 3: Reasons for going to Government Hospital and Health Clinics, and age at diagnosis of Homozygous HbE patients

Patient No.	Age (years)	Sex	*Reasons
1E	38	M	Thal screening
2E	37	F	Anemia
3E-F	41	M	Thal screening
3E-D1	14	F	Thal screening
3E-D2	10	F	Thal screening
4E	28	F	Anemia
5E	44	F	Anemia

*Reasons for going to Government Hospital and Health Clinics
F: Female; NIDDM: Non insulin dependent diabetes mellitus; M: Male; HPT: Hypertension; Thal screening: Thalassemia screening

Table 2 shows the reasons for going to Hospital and Health Clinics, and age at detection of HbE β-thalassemia patients. Age at detection of HbE β-thalassemia ranged from 1 to 70 years. Patient 3A presented with non insulin dependent diabetes mellitus and hypertension, while patient 5A presented with fever and jaundice. Patient 2A was diagnosed as HbE β-thalassemia during ANC when she had anemia in pregnancy. Patient 2A-S (son of 2A) had HbE β-thalassemia like his mother.

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Table 3 shows the reasons for going to Hospital and Health Clinics, and age at detection of homozygous HbE. Age at detection of the 7 patients with homozygous HbE range from 10 to 44 years. Patient 1E was screened for thalassemia because his wife was a β-thalassemia carrier. Three patients (2E, 4E, and 5E) presented with anemia during pregnancy. Homozygous HbE was detected in 3 family members (3E-F [father], 3E-D1 [daughter 1), 3E-D2 [daughter 2]) during thalassemia screening. The mother of the family (3E-M) was a HbE carrier.

Discussion

Perlis had a population of about 227,000 as of 2010. The ethnic composition was Malay: 79.7%, Chinese: 9.6%, Indian: 1.25%, and others: 9.4%. Perlis had slightly more females than males (1: 0.97) [3]. In our study, a majority of the patients were Malays (91.4%). Female patients outnumbered male patients in the ratio of 3.5:1. This could be due to the fact that 75.7% of the female patients were of childbearing age (17 - 40 years) and a majority of them were at the Health Clinics and hospital for their ANC. These female patients had anemia in pregnancy.

The screen tests used for Hb analysis in this study were FBC, HPLC and gel electrophoresis. Hb analysis using our screen tests were within normal limits in 42 out of 499 (8.4%) patients. About 48.9% of the patients had normal Hb subtypes, but was unable to rule out α-thalassemia and normal HbA2 β-thalassemia. These patients had MCV < 80 fl or/and MCH <27 pg. Molecular studies for α-thalassemia had to be carried out on this group of patients. Hb E trait and β-thalassemia trait was found with a frequency of 19.3% and 14.6% respectively. The other hemoglobinopathies detected were HbH disease (1.8%), HbCS (1.6%), HbE β-thalassemia (1.4%), homozygous HbE (1.4%), and other types of hemoglobinopathies (2.6%). FBP done at the Hospital and Health Clinics showed that all these patients had microcytic hypochromic anemia except for some who went for thalassemia screening.

The three common thalassemic diseases detected were HbH disease, HbE β-thalassemia, and homozygous HbE. HbE β-thalassemia was detected in 7 Malay patients, two pediatric patients at age 1 year, and five adults at ages 19-70 years. Compound heterozygous for HbE β-thalassemia is responsible for about half of the severe β-thalassemia, and clinically it is very diverse in nature due to both genetic and environmental factors. The disorder may range from mild asymptomatic anemia to a life threatening disorder requiring transfusions throughout life. Modifying genetic factors such as co-inheritance of a mild α-thalassemia allele, β-thalassemia, and polymorphisms associated with increased *in vivo* synthesis of fetal Hb (HbF) may result in a milder clinical course of HbE β-thalassemia [8]. The mild clinical course of HbE β-thalassemia is reflected by fewer diagnosed cases than the number expected based on the Hardy-Weinberg equation [9]. The five adult patients detected of having HbE-β thalassemia (Patients 1A, 2A, 3A, 5A and 6A) probably had the mild phenotype with asymptomatic anemia, as they did not require any lifelong blood transfusion. Patients who had the 'severe' form of HbE β-thalassemia with lifelong, transfusion dependent anemia would have been detected by one year of age.

Homozygous HbE was detected in seven Malay patients (aged 10-44 years) during thalassemia screening or presentation with anemia in pregnancy. Tachavanich *et al*., [10] found that HbE homozygotes were clinically benign, did not require any blood transfusion and had no hepatosplenomegaly. However, parents who are homozygous HbE and β-thalassemia carrier have a 50% chance of having a child with HbE β-thalasemia. HbH disease is characterized by moderate, variably compensated hemolytic anemia that may present with clinical symptoms during a period of physiological stress such as infections, pregnancy, or surgery. HbH disease was detected in 9 adult patients (age 22 to 76 years) when they had anemia or during thalassemia screening.

Thalassemia can be prevented through educational and screening programmes, and provision of prenatal diagnostic services. Thalassemia carrier screening programmes could be mandatory or voluntary depending on the country. Thalassemia carrier couples can decide to go for prenatal diagnosis after conception and elect for termination of pregnancy if the fetus is affected with β-thalassemia major. The incidence of β-thalassemia major decreased significantly after the introduction of mandatory premarital screening programs in Cyprus, from 51 affected births in 1973 to no affected births occurring between 2002 and 2007 [11]. Antenatal screening programmes in Taiwan and Guangdong, China have also resulted in a significant reduction in the incidence of β-thalassemia major [12, 13]. The provision of thalassemia screening programmes are also affected by cultural, social and religious beliefs. Prenatal diagnosis is not offered to couples in some countries, as pregnancy termination is forbidden on

religious grounds [14]. In Malaysia, thalassemia education has been provided through the mass media, lectures presented to the general public, posters and pamphlets, training of health personels, and provision of genetic counseling for thalassemia carriers. Thalassemia carrier screening is not mandatory and available free of charge in various government hospitals and health clinics throughout the country [15, 16].

Conclusion

Using our screen tests (FBC, HPLC and AGE), the two most common hemoglobinopathies detected among patients in a government hospital and Health Clinics in the state of Perlis, Malaysia were HbE trait (19.3%) and β-thalassemia trait (14.6%). We also detected compound heterozygous for HbE-β thalassemia when they were adults as they did not require any transfusion for life. With thalassemia education and carrier screening programmes, thalassemia would probably be a preventable disease in Malaysia in the future.

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