ORIGINAL ARTICLE

Screening of Concurrent α-Thalassaemia 1 in β-Thalassaemia Carriers

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Summary

Thalassaemia is an inherited blood disorder and is a significant public health problem in Malaysia, with many not knowing they carry the gene for thalassaemia. The two major forms are alpha and beta thalassaemia. An individual can co-inherit both the alpha and beta thalassaemia genes. This study determined the frequency of concurrent carriers of alpha thalassaemia in 231 beta thalassaemia carriers. Gap-PCR was done on extracted DNA of the beta thalassaemia samples to check for alpha thalassaemia 1 molecular defect. Eight (3.5%) samples were found to have concurrently inherited the alpha thalassaemia 1 ($--^{SEA}$) deletion. The significant carrier rate for alpha thalassaemia 1 indicates the need for the implementation of DNA analysis to complement thalassaemia screening in high risk populations.

Key Words: Thalassaemia, Concurrent carriers, Screening, Gene interaction

Introduction

Thalassaemia is a hemoglobin disorder characterized by the absence or reduced synthesis of globin chains, α, β, γ, δ, ε and ζ of human Hb. The two main types of thalassaemia are α- and β-thalassaemia.

Disorders of globin chain synthesis, including thalassaemia, are common in Malaysia and constitute a significant public health problem. There is no national registry to determine the number of thalassaemia patients, but the Health Ministry recently announced that a census will be started to register all thalassaemia patients and carriers in the country. Three to five percent of Malaysians are estimated to be thalassaemia carriers, which amounts to between 600,000 and one million people[1].

Alpha-thalassaemia is the most common hemoglobin disorder in the world. Deletions of either one (α-thalassaemia 2) or both (α-thalassaemia 1) α-globin genes on chromosome 16 account for over 95% of α-thalassaemia cases[2].

In Southeast Asia, the form of mutation in α-thalassaemia 1 carriers is most commonly the SEA deletion ($--^{SEA}$). Couples who are carriers of α-thalassaemia 1 are at risk of producing a Hb Bart's hydrops fetalis offspring that usually dies in utero at the third trimester of pregnancy or shortly after birth[2].

Phenotypically there are two forms of β-thalassaemia: β^0 – no β globin chain synthesis, and β^+ - with some β globin chain synthesis, clinically presenting as a trait (β^+ or β^0), thalassaemia intermedia (β^+/β^+ or β^+/β^0) and β-thalassaemia major (β^0/β^0).

Beta-thalassaemia major patients are transfusion dependent. Without transfusion, death occurs in the

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first few years of life. As a result of repeated blood transfusions iron overload will set in; desferrioxamine, an iron-chelating agent, has to be given by continuous infusion via pump and is expensive. Without chelation, they will die due to iron overload[3]. Cure being possible in those who underwent an allogenic transplant with an HLA matched donor with stem cells sourced from bone marrow, peripheral blood or umbilical cord.

Classical β-thalassaemia trait have hypochromic microcytic, red cell indices, and a raised Hb A2 with values >4% when measured by high performance liquid chromatography (HPLC)[4]. Red blood cells in a-thalassaemia 1 are also hypochromic and microcytic, and are presumptively identified by positive H inclusion test and normal HbA2 level. Confirmation of α-thalassaemia 1 requires DNA studies.

Despite the same genotype, there is a remarkable variability in severity of the clinical presentation of β-thalassaemia. Studies indicate that one of the genetic factors that influence the severity of β-thalassaemia is the coinheritance of the a-thalassaemia gene.

Studies indicate that interactions between α- and β-thalassaemia must be considered when investigating moderate to severe hypochromic microcytic anemia of uncertain cause in adult patients from areas with a high prevalence of globin gene mutations, and that the co-inheritance of both α and β-thalassaemia does indeed occur in these areas, confirmed by PCR-based techniques[5,6].

Thus, the presence of β-thalassaemia trait does not exclude the simultaneous presence of α-thalassaemia 1. Failure to detect both abnormalities may lead to a failure to predict Hb Bart's hydrops fetalis when one partner has α-thalassaemia 1 and the other has both α-thalassaemia 1 and β-thalassaemia trait[7]. The laboratory investigation of concurrent α-thalassaemia and β-thalassaemia is important for the identification of people who are of reproductive age, and are potential parents to offspring with Hb Bart's hydrops fetalis and β-thalassaemia major.

In view of the recent announcement of a national screening program by the Ministry of Health, the result of this study will indicate whether there is a need to screen for α-thalassaemia 1 in carriers with β-thalassaemia to prevent severe forms of thalassaemia in Malaysia.

Materials and Methods

Two hundred and thirty one β-thalassaemia samples were studied. These cases were identified as classical β-thalassaemia carriers by low MCV<80fL, MCH<27pg and a raised HbA2>4%. The numbers of samples collected were appropriate statistically according to the table given by World Health Organization[8], with an assumption that 10% of the β-thalassaemia carriers concurrently carry the α-thalassaemia 1 gene, according to previous studies done in Hong Kong[5] and Guang-Dong district in China[9].

Approval for this study was obtained from the Medical Ethics Committee at Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The samples were analyzed using conventional hematological methods. For screening of thalassaemia and hemoglobinopathies, the first step is a scrutiny of the red cell indices using a cut-off value of MCV <80fL or an MCH <27pg[7]. Automated blood counts and red blood cell indices were generated on an automated blood counter (Celldyne 1700, Abbott Laboratories, Abbott Park, IL, USA).

The quantitation of Hb subtypes was by high performance liquid chromatography (HPLC) (Variant, Bio-Rad, Hercules, CA, USA). Accurate quantification of HbA2 for the diagnosis of a β-thalassaemia trait is imperative, and this is possible via that use of HPLC. Raised HbA2 of >4% is indicative of b-thalassaemia trait when the Hb is quantified by HPLC[4].

DNA was extracted from blood leucocytes using commercial DNA extraction kits from Qiagen (Qiagen Inc., Valencia, CA, USA). Gap-PCR was used extensively for diagnosis of α-thalassaemia 1. This method, based on methods by Winichagoon P. *et al*, 1995 (10), was applied to detect the specific deletion of 20kb causing the molecular defect. The primers A4 (5'-GGGGCGCCTTGGGGAGGTTC-3') and A9 (5'-ATATATGGGTCTGGAAGTGTATC-3') are specific for a-thal ($--^{SEA}$) deletion, and the primers A4 and A1B (5'-GTTCCCTGAGCCCCGACACG-3') were used for normal alleles.

In the gap-PCR reaction, the PCR product was electrophorised on 1.5% agarose gel, stained with ethidium bromide solution and studied on a UV transilluminator. Separation based on fragment lengths allowed the identification of the a-thalassaemia 1 gene.

Results

Two hundred and thirty one b-thalassaemia samples were studied. There were 118 Chinese (51.1%), 99 Malays (42.9%) and 14 of other races (6.1%) comprising of five Orang Asli, three Thai, three Filipino, one Indian, one Dusun and one of Indian and Thai mixed heritage.

In the 231 β-thalassaemia samples, 8 (3.5%) were found to be concurrent carriers for α-thalassaemia 1. Six (75.0%) were Chinese and 2 (25%) were Malays.

Discussion

Thalassaemias are common among Southeast Asians and Southern Chinese. Due to the coexistence of several thalassaemia and related genes in the same area, Southeast Asia witnesses the most complex thalassaemia syndromes unparalleled by other parts of the world[2].

The clinical presentations of a carrier with classical β-thalassaemia and α-thalassaemia 1 are very similar, being asymptomatic, with both presenting with microcytosis and hypochromia.

Thalassaemia screening begins with: scrutiny of full blood count; measuring levels of different Hb subtypes by HPLC; and measuring serum ferritin level to rule out iron deficiency. Presumptive identification of α-thalassaemia 1 is made when MCV<80fL, MCH<27pg, HbA_2<4.0%, serum ferritin level is normal, an H inclusion test is positive and an increased ζ-globin chain level.

A positive H inclusion test and ζ-globin assay might indicate α-thalassaemia 1. The H inclusion test is tedious and laborious. A negative result does not rule out α-thalassaemia 1. The ζ-globin assay by enzyme-linked immunoabsorbent assay (ELISA) appears promising as a screen test[11,12]. Gap-PCR, a simple PCR based DNA study accurately identifies α-thalassaemia 1 molecular defect.

Interactions of different mutations have been reported[5,6,13]. In this study, the rate of co-inheritance of α-thalassaemia 1 ($--^{SEA}$) deletion among β-thalassaemia carriers was found to be 3.5%. This result indicates the need to identify both α- and β-thalassaemia genes so as to prevent the birth of Hb Bart's hydrops fetalis and b-thal major.

Other studies reported the frequency of α-thalassaemia 1 ($--^{SEA}$) deletion in β-thalassaemia carriers as 9.1% (8 in 88) in Hong Kong (5) and 8.6% (43 in 500) in GuangDong district, China[9]. There have been no published reports on the presence of concurrent carriers for α- and β-thalassaemia in Malaysia.

In year 2005, as Malaysia moves towards the implementation of a national thalassaemia screening program, it is important to include also the identification of α-thalassaemia 1 in carriers of β-thalassaemia to prevent the births of Hb Bart's hydrops fetalis.

The success in Sardinia, Italy and elsewhere in reducing the prevalence of thalassaemia major by genetic counseling shows that screening can have a major impact in communities in which the thalassaemias are common[14,15].

Table I: Concurrent alpha thal 1 in beta-thalassaemia carriers

Race	Samples	Approximate %	Concurrent carriers	% in respective population
Malays	99	42.9	2	2.0
Chinese	118	51.1	6	5.1
Others	14	6.1	0	0
Total	**231**	**100**	**8**	**3.5**

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