ABSTRACT

Thalassemia is a heterogenous group of monogenic hereditary disorders present in a restricted geographic area known as the "thalassemic belt", stretching from the Meditteranean, continuing eastward through the Middle-East, the Indian Subcontinent and South-East Asia reaching the South-West Pacific (Melanesia) region. It is due to mutations which reduce the synthesis of either the α globin (α thalassemia) or β globin (β thalassemia) chain leading to a decrease in blood Hb. The resulting anemia can vary from very mild, needing no treatment, to severe, needing life long rounds of transfusions.

Blood transfusions which presently is the main treatment of thalassemia is unsatisfactory because it does not lead to permanent cure and may even lead to complications. Thus the strategy to deal with this disorders is of necessity preventive, i.e. to prevent the birth of babies who get the heredity of severe clinical thalassemia and to lower the incidence of thalassemia.

The severity of the clinical symptoms depend mainly on the type and the zygocity (heterozygous or homozygous) of the mutation. Interaction with other hemoglobinopathy may aggravate clinical symptoms, such as in cases of HbE / Thal β and Hb Constant Spring (CS) / Thal α double heterozygotes.

Heterozygous individuals are often missed by conventional hematological laboratory tests. The presence (or absence) of the thalassemia causing mutation in these individuals can only be detected by molecular methods. It is also known that the incidence of the various thalassemic mutations may vary from one geographic region to another.

Indonesian's geographic location lies within the thalassemia belt. It has been estimated that in Indonesia around 4.6 million individuals carry the Thal β gene, around 700,000 carry the Thal α gene and around 6.2 million carry the HbE gene. The incidence of the HbCS gene is as yet unknown. Although estimates were given, these were based on studies covering limited areas within Indonesia, and studies covering more areas are still needed in order to obtain the whole picture.

The present study is limited to patients attending the Dr Sutomo General Hospital, Surabaya. They came from Surabaya City and surrounding areas. Beta thalassemia cases came from patients attending the Hematology Clinic, Dept of Pediatrics. Since α thalassemia cases were not available from the Hematolgy Clinic, case finding was attempted by examining anemic pregnant mothers presenting for their first visit to the Maternity Clinic, Dept of Obgyn.
Thalassemia β mutations were detected by PCR-DGGE, followed by DNA sequencing if necessary. Thalassemia α mutations were detected using AS-PCR, HbCS was detected by PCR-Dot blot hybridization.

During the study 75 β thalassemic children (one ethnic Chinese and 74 ethnic Javanese), comprising a total of 75 x 2 = 150 chromosomes were examined. The result was as follows (1) Cd26 GAG→AAG (HbE) : 73/150 (48%); (2) IVS 1 nt 5 G→C : 37/150 (25%); (3) IVS 1 nt 1 G→T : 10/150 (7%); (4) IVS 2 nt 654 C→T : 8/150 (5%); (5) Cd 41-42, del CTTT : 6/150 (4%); (6) Cd15 G→A : 3/150 (2%); (7) Cd30 G→C : 3/150 (2%) and (8) undetected by PCR-DGGE : 10/150 (7%). Among the 10 undetected cases, one mutation : nt -28 ATAAAA →ATAGAA was detected by DNA sequencing, leaving nine undetected cases.

Among the 75 β thalassemic children one was homozygous for the HbE gene and 71 were HbE/ Thal β double heterozygotes, leaving only three were not exhibiting the HbE gene. This result emphasize the high incidence of the HbE gene and HbE/ Thal β double heterozygotes, i.e. 72/75 (96%) and 71/75 (95%) respectively. One interesting finding in this study was the fact that the one child homozygous for the HbE gene presented with severe anemia (Hb 4.1 g/dl) which is unusual, since HbE homozygous usually present with only mild anemia. It is suspected that another mutation might be present in this child.

The incidence of the different mutations found in this study is probably an underestimate since all these children, exhibited moderate to severe anemia (Hb 4.1-6.5 g/dl), and all except one were double heterozygotes.

In order to investigate whether the local mutants found in this study relate with those found in surrounding regions haplotype determinations were done on the β globin gene cluster using the following restriction endonucleases : Hinc II, Hind III, Hinf I, Ava II, Hpa I and Bam HI. The Cd 26 GAG→AAG (HbE) and IVS 1 nt 5 G→C were chosen because together they represent the more frequent mutation (73%) and IVS 2 nt 654 C→T were chosen representing the less frequent mutation (5%). The result was as follows : 50% (5/10) Cd26 GAG→AAG (HbE) mutants expressed the same haplotype (+- - - - + + + + + and - - - - - + + + + +) as that found in South China and South East Asia, 70% (7/10) IVS 1 nt 5 G→C mutants expressed the same haplotype (+ - - - - + + + + +) as that found in South West Pacific (Melanesia) and 20%(2/10) has (+ - - - - + - + + +) as that found in India Asia , 100% (8/8) IVS 2 nt 654 C→T mutants showed the same haplotype (+ - - - - + + + + + and + - - - - - - + + +) as that found in either in South East Asia or South China. These finding probably reflects the genetic heterogeneity of the Indonesia population.

The result of case finding on 125 pregnant mothers were as follows : thal α1 ($-\alpha^{17,5}$) : 2,4% (3/125), HbCS 0% (0/125). No thal α 2 ($-\alpha^{3,7}$ or $-\alpha^{4,2}$) were found in this study due to technical failure of AS-PCR to detect these mutation.

Key words : genetic variation, α thalassemia, β thalassemia, HbE, HbCS, β globin gene cluster, haplotype.