Evaluation of Heterozygous Hb E and Its Interaction with Deletional Alpha Thalassaemia in Kelantan

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Haemoglobin E (Hb E) is a variant of structurally abnormal haemoglobin that can be found very commonly in the Asian countries particularly the Southeast Asian [1]. Alpha thalassaemia is a red cell disorder which is caused by deletion or mutation of one or more of the four alpha globin genes leading to absence or decrease in production of alpha globin peptides [2]. This disorder is far more common in South East Asian regions and in Malaysia itself, and the gene frequency is about 4.1% [2]. The interactions of Hb E and alpha thalassaemia are evident in Kelantan which is bordered by southern Thailand. Using capillary electrophoresis (CE), a reduction of Hb E level is noticed as compared to Hb E heterozygotes. DNA analysis should be done to determine the presence of concurrent alpha thalassaemia variant. This study was done to evaluate haematological parameters using automated blood counters, morphology of red cells, Hb separation and quantitation of Hb fractions using CE and molecular analysis for alpha thalassemia. The study also aimed to discover cut off point of Hb E level in heterozygous Hb E patients with concurrent deletional alpha thalassaemia by CE.

A preliminary data analysis involving 59 blood samples were enrolled in a cross sectional study. Samples were obtained from various places in Kelantan state for the investigation of anaemia, hypochromic microcytic red cells and family screening for thalassaemia. Each sample was tested for full blood count, blood smear, CE and DNA analysis. Data were analyzed using one way ANNOVA. The mean age was 18.76 (13.68) years old and majority were female patients (67.8%). Among all patients, 88.1% was Malay while the rest were non-Malays including Chinese, Siamese and aborigines (Orang Asli). 16 samples (27.1%) were Hb E heterozygote with deletional alpha thalassaemia 3.7 gene deletion, 13 samples (22.0%) were Hb E heterozygote with double gene deletions (South East Asian (SEA) type) and 1

sample (1.7%) was Hb E heterozygote with compound heterozygous 4.2 and 3.7 gene deletions. All six haematological parameters (RBC, Hb, MCV, MCH, RDW and Hb E) were compared between all groups of Hb E heterozygote with concurrent deletional alpha thalassaemia variants (refer table 1). The results showed that there were no significant differences between the variables except for Hb E ($p < .001$). Mean for Hb E level in group Hb E heterozygote with deletional alpha thalassaemia 3.7 single gene deletion and double gene deletion SEA type is 21.42 (1.45) and 16.37 (0.74) respectively.

Table 1: Comparison of six haematological parameters between all groups of Hb E heterozygote involved in this study.

Variables	Means
RBC (x 10 12/l)	
Hb E with no deletional alpha thalassaemia Hb E alpha (3.7 gene deletion) Hb E alpha (SEA gene deletion)	4.65(0.65) 4.77(0.73) 5.11(0.57)
Hb (g/dl)	
Hb E with no deletional alpha thalassaemia	10.19(2.40)
Hb E alpha (3.7 gene deletion)	10.97(1.89)
Hb E alpha (SEA gene deletion)	10.59(1.14)
MCV (f/L)	
Hb E with no deletional alpha thalassaemia	68.27(9.90)
Hb E alpha (3.7 gene deletion)	70.98(8.12)
Hb E alpha (SEA gene deletion)	63.32(4.36)
MCH (pg)	
Hb E with no deletional alpha thalassaemia	21.95(4.21)
Hb E alpha (3.7 gene deletion)	23.08(2.70)
Hb E alpha (SEA gene deletion)	20.73(1.29)
RDW (%)	
Hb E with no deletional alpha thalassaemia	14.42(6.35)
Hb E alpha (3.7 gene deletion)	14.19(7.14)
Hb E alpha (SEA gene deletion)	15.08 (4.95)
Hb E level (%)	
Hb E with no deletional alpha thalassaemia	21.50(2.55)
Hb E alpha (3.7 gene deletion)	21.42(1.45)
Hb E alpha (SEA gene deletion)	16.37(0.74)

As a conclusion, Hb E heterozygote with deletional alpha thalassaemia had lower Hb E level and preliminary diagnosis can be predicted using automated capillary electrophoresis. It is recommended to confirm alpha thalassaemia variant by molecular analysis.

Keywords: Hb E, alpha thalassaemia, capillary electrophoresis, DNA analysis

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