UNIVERSITI TEKNOLOGI MARA

DETECTION OF BETA-GLOBIN GENE MUTATIONS IN MALAYSIA: COMPARISON BETWEEN MARMS AND FTH METHODS

By

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DECLARATION

"I hereby declare that this thesis is my original work and has not been submitted previously or currently for any other degree at UiTM or any other institutions."

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ABSTRACT

Beta thalassaemia is one of the common inherited blood disorders worldwide. This disease occurred due to mutations such as substitution of nucleotide, frameshift mutation, minor deletions and rarely due to large major deletions. Nowadays, many techniques were applied for mutation analysis and multiplex-amplification refractory mutation system (MARMS) is an available method being used for diagnosis of common beta globin gene mutations, particularly in patients diagnosed in UKMMC. However, it is labor-intensive and time-consuming especially when detecting numerous common beta-globin gene mutations. Recently, the flow-through hybridization (FTH) method was introduced. Thus, the aim of this study was to compare the detection of beta globin gene mutations using MARMS and FTH techniques in patients of UKMMC. A total of 100 samples of EDTA blood were obtained from patients diagnosed as thalassaemic patients and were screened with MARMS. A total of 56 specimens screened by MARMS were chosen for FTH assay. From the 56 cases that were successfully detected by both methods, 40 samples had similar results for detection of mutations. In addition, the FTH assay managed to show 12 samples with additional mutations including the 45 Kb deletion, 619bp deletion, Cap+1 and Poly A. However, two samples were not detected in FTH but were detected with MARMS (Cd 8/9). For genotyping result, 43 samples were detected as heterozygous in FTH while 44 samples were reported as heterozygous in MARMS. The discrepancies observed were due to the primers not included in the respective assays. Hence, there is possibility of using FTH which is much simpler and rapid method for detection of vast common beta globin gene mutations.

Keywords: beta globin gene mutations, MARMS, FTH.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Mutation on beta globin chains result in improper synthesis of haemoglobin which is known as haemoglobinopathies and beta-thalassaemia. Beta-thalassaemia is associated with mutation in the beta globin gene at chromosome 11 (Kulkarni et al., 2013). As for haemoglobinopathies, there are a several types of abnormal haemoglobins; haemoglobin E (HbE), haemoglobin S (HbS) and haemoglobin Lepore. Hb E is an important and common beta globin chain variant in Southeast Asian involving Thailand, Laos, and Cambodia (Kohne, 2011 and George in 2013).

Therefore, proper routine screening test for beta globin gene mutations is crucial. Screening of the full blood picture especially on the red blood cells indices is the first step for identification of the beta-globin chain diseases. An individual having this disease will show low red blood cell count, low mean corpuscular volume (MCV) and low mean cell hemoglobin (MCH) (Old, 2003). In Universiti Kebangsaan Malaysia Medical Centre (UKMMC), capillary electrophoresis (CE) is used for screening of beta globin chain diseases. However, this technique has its limitation because it only involves separation of the various types of hemoglobin within the sample (Alauddin et al., 2012).

With the current advancement in technology, confirmation can now be done using molecular techniques. Among the popular molecular method commonly used today is the polymerase chain reaction (PCR). PCR is a definitive method for diagnosis of beta globin gene mutations since it will be able to detect mutations that occurred in the beta globin gene. Molecular analysis such as multiplex-arms refractory mutation system (MARMS) is a current method for detection of beta globin gene mutation in Malaysia (George et al., 2012). However, this technique is tedious and time consuming