DIAGNOSIS OF FRAGILE X SYNDROME BY USING CYTOGENETIC AND MOLECULAR APPROACHES

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ABSTRACT

The study of fragile X syndrome diagnostic tests was conducted at the Human Genome Center (HGC), Hospital University Science Malaysia (HUSM). In this study, two diagnostic tests were investigated, namely cytogenetic test and molecular test. Cytogenetic test employed standard method of karyotyping to investigate the structural abnormalities of chromosome X. From the karyotypes produced, abnormal fragile site was detected on the chromosome X of one (Sample 6) out of seven samples (14.3%). The presence of fragile site on chromosome X indicates that the patient is affected by fragile X syndrome. Molecular test was based on the study of the gene that causes this syndrome, namely Fragile X Mental Retardation-1 (FMR1) gene. An extension of CGG triplet repeat region at the 5' end of FMR1 gene can cause fragile X syndrome. The focus of molecular test was to investigate the $(CGG)_n$ variant in the 7 samples using PCR technique. With the PCR analysis, normal FMR1 allele was detected in six out of seven samples (Sample 1 to 7 except Sample 6). The CGG repeats number of these normal samples ranges between 8 and 30 repeats. In Sample 6, no amplified fragment was generated by PCR in the first trial. However, after optimization, a DNA fragment with estimated size 850bp was amplified. The number of CGG repeats of this fragment was 215 repeats. This showed that the particular patient had expanded CGG alleles on FMR1 gene and he was diagnosed as fragile X syndrome positive patient. Molecular test diagnostic result was comparable with the diagnostic result of cytogenetic test. Both tests revealed that among the seven samples, only one sample (Sample 6) was fragile X syndrome positive.

ABSTRAK

Kajian untuk mendiagnos penyakit sindrom Fragile X ini dijalankan di Pusat Genom Manusia (PGM), Hospital Universiti Sains Malaysia (HUSM). Dalam kajian ini, dua ujian diagnos telah dijalankan iaitu ujian sitogenetik dan ujian molekular. Ujian sitogenetik menggunakan kaedah karyotyping untuk mengenalpasti ketidaknormalan kromosom seseorang individu. Daripada karyotype yang dihasilkan, kawasan rapuh berjaya dijumpai pada kromosom X dalam satu sampel di kalangan kesemua tujuh sampel yang terlibat (14.3%). Kehadiran kawasan rapuh pada kromosom X mengesahkan bahawa sampel itu adalah pesakit sindrom Fragile X. Ujian molekular pula berdasarkan kajian terhadap gen yang menyebabkan sindrom Fragile X iaitu gen FMR1 (Fragile X Mental Retardation 1 gene). Punca sindrom Fragile X adalah disebabkan oleh pemanjangan tidak terkawal pada satu siri rangkaian ulangan trinukleotide CGG pada kawasan 5' ekson 1 gen FMR1. Ujian molekular memfokus kepada siasatan variasi trinukleotida $(CGG)_n$ dengan menggunakan tindak balas rantai polimerase (PCR). Daripada saiz produk PCR, didapati bahawa semua sampel (kecuali Sampel 6) mempunyai alel normal di mana bilangan ulangan motif CGGnya berada dalam lingkungan 8 hingga 30. Untuk Sampel 6, tiada produk PCR yang berjaya dihasilkan dalam cubaan pertama. Namun begitu, dalam cubaan kedua, produk PCR dengan saiz jangkaan sebesar 850bp berjaya diperolehi. Bilangan ulangan motif trinuleotida CGGnya adalah sebanyak 215. Pemanjangan motif CGG sehingga melebihi 200 ulangan ini mengesahkan bahawa Sampel 6 ialah pesakit sindrom Fragile X positif. Kedua-dua ujian molekular dan sitogenetik menghasilkan