An Alternative Method for Rapid Detection of Alpha Thalassaemia Variants in Malaysia using Droplet Digital PCR

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Globally, α-thalassaemia is a highly prevalent disease. In Malaysia, this disorder is a well-known public health problem [1]. The three most common deletional α-thalassaemia found in this region include $--^{SEA}$ deletion, $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions [2]. The prevalence rate of triplication alpha cases such as $\alpha\alpha\alpha^{anti3.7}$ and $\alpha\alpha\alpha^{anti4.2}$ is unknown in Malaysia although it plays a pivotal role in exacerbating the clinical phenotypes in beta thalassaemia carriers [3]. Therefore, the purpose of this study was to design an assay for the detection of triplications and common deletional alpha thalassaemia using droplet digital PCR (ddPCR). Copy number changes were analysed using Quanta-Soft™ software version 1.6.6 after performing ddPCR. Sensitivity and validation analysis were also performed on the DNA samples. The changes in copy number changes (common deletions, duplications and triplications) in the alpha globin gene has been quantitatively detected using ddPCR. For the samples validation as determined by ddPCR, the mean copy number values for αα/αα are 2.0275±0.0177 (HS-40), 1.8175±0.0389 (HBA2), 2.0450±0.0848 (HB 3.7), 2.0050±0.0000 (HBA1). For $-\alpha^{3.7}/--^{SEA}$, the mean copy number values are 2.0225±0.2180 (HS-40), 0.9325±0.1213 (HBA2), 0 (HB 3.7), 0.9984±0.1333 (HBA1). As for $–\alpha^{4.2}/--^{SEA}$, the mean copy number values are 1.9350 (HS-40), 0 (HBA2), 0.7945 (HB 3.7), 0.8480 (HBA1). The mean copy number values for $--^{SEA}/\alpha\alpha$ samples are 1.9067±0.1327 (HS-40), 0.8164±0.0364 (HBA2), 0.8920±0.0434 (HB 3.7), 0.9148±0.0338 (HBA1) respectively. This study has found that the use of ddPCR is convenient as it allows direct quantification without the requirement of a calibration curve unlike qPCR [4]. Secondly, this study also showed that ddPCR is accurate and precise in the detection of alpha thalassaemia deletions and triplications based on the gene dosages using

absolute quantification. In addition, the non-requirement of post-PCR work has minimised the risk of PCR carryover contamination. Thirdly, ddPCR saves time with less turnaround time and minimise the labour work required as compared to techniques such as MLPA which requires DNA denaturation and hybridisation reaction on day 1 while ligation and PCR reaction on day 2. Fourthly, this study found that the detection of α-thalassaemia using ddPCR is sensitive. DNA samples with low concentration as low as 1 ng were able to be detected for α-thalassaemia using ddPCR. The ability to detect minute amount of DNA concentration is crucial particularly in the diagnosing of the lethal HbH hydrops foetalis during the neonatal stage in α-thalassaemia. In conclusion, this is an alternative method (ddPCR) that can be employed for rapid detection of alpha thalassaemia variants in Malaysia.

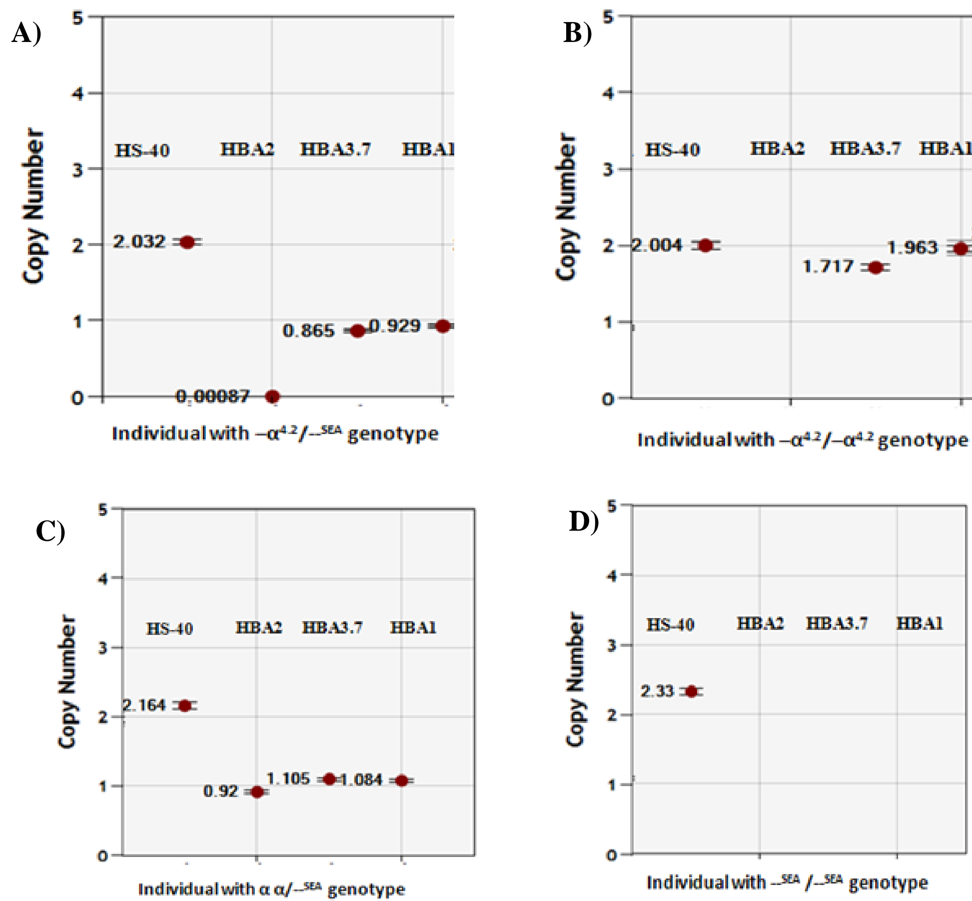

Fig 1: **The ratio of DNA copy number for 4 different copy number assays (HS-40,HBA2, HBA3.7 and HBA1)** **A)** Individual with $–\alpha^{4.2}$/--^{SEA} genotype **B)** Individual with $–\alpha^{4.2}$/-$\alpha^{4.2}$genotype **C)** Individual with --^{SEA} /αα genotype **D)** Individual with --^{SEA} /--^{SEA} genotype.

Keywords: digital droplet PCR, alpha thalassaemia, Malaysia, method, rapid

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