Line Probe Assay for Detection of Alpha Thalassemia: A Pilot Study

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ABSTRACT
This pilot study was initiated with a view to find α thalassemia genotypes on de-identified samples from patients diagnosed with anaemia at the Centre for Advanced Biomedical research and Innovation (CABRI) at Gulf Medical University (GMU) in June 2015. Amplified DNA from the samples was probed for mutations using a line probe assay. Results obtained are presented. The study has shown the 3.7 single gene deletion in three cases, and α 2 IVS1 (-5nt) mutation seen in one case suggesting these cases have α + thalassemia. One sample showed wild type α 2 Poly A missing along with the α 2 poly a-1 (AATAAA>AATAAG) mutation with a suggestive diagnosis of HbH disease. A SEA double gene deletion was seen in one case suggesting α 0-thalassemia. Further studies are being carried out to enhance the data base.

Keywords: Alpha Thalassemia, anaemia, line probe assay
INTRODUCTION

Anaemia in children has been reported as high as 36.1% in the UAE. The causes of anaemia have been defined primarily as iron deficiency (36.0%), beta-thalassaemia (8.7%) with an unknown percentage with Alpha Thalassemia\(^1\). Alpha-Thalassemia mutations affect up to 5% of the world’s population\(^2\). Studies at the GMC hospital in 2014 have shown anaemia to be predominantly Normocytic Normochromic (50.95%) followed by Microcytic Hypochromic (47.14%). Macrocytic anaemia was seen only in 1.9% of the cases examined. This pilot study was carried out to identify various alpha thalassemia genotypes in de-identified samples from patients with anaemia.

Production of α-globin chains is regulated by four gene loci. Depending on the number of gene foci affected four α thalassemia types have been described. When three functional alpha genes are present, patient will have a ‘silent’ α thalassemia. The α thalassemia trait usually results from deletion or dysfunction of two α globin genes, the homozygous deletion being the α\(^+\) variant (down regulated expression from a single chromosome, both chromosomes being affected) and the heterozygous being the α\(^0\) variant (completely abolishing expression from a chromosome). In α\(^-\) or α\(^0\) thalassemia, the blood smear shows microcytosis, hypochromasia, and slight anisocytosis and poikilocytosis. Deletion or dysfunction of three α-globin alleles results in HbH disease and presents with mild-to-moderate (rarely severe) microcytic hypochromic haemolytic anaemia with hepatosplenomegaly. On electrophoresis, about 3% to 30% of the total haemoglobin is HbH. When all four genes are affected, α-chains are absent, and tetramers of γ-chains called Hb Bart, form in the foetus usually resulting in hydrops fetalis and intrauterine death\(^3\).

MATERIAL AND METHODS

This study was carried out at CABRI, GMU. Ten de-identified cases of microcytic hypochromic anaemia, showing target cells on peripheral smear, with HbA2 levels less than 2.8% were selected for the study.

Complete blood counts were done on a validated Sysmex hematology analyser (2000i). HPLC was carried out using a Tosoh HLC-723G7 which is a fully automated high performance liquid chromatography instrument–reagent system that rapidly and precisely separates haemoglobins found naturally in blood.

The procedure for the strip assay includes three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers and (3) hybridization of amplification products to test strips containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. DNA extraction was carried out using the DTAB/CTAB technique. Extracted DNA Purity was assessed using a Nanodrop. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and colour substrates.

The α thalassemia assay covers 21 α-globin mutations: 3.7 single gene deletion, 4.2 single gene deletion, MED double gene deletion, SEA double gene deletion, THAI double gene deletion, FIL double gene deletion, 20.5 kb double gene deletion, anti-3.7 gene triplication, α1 cd 14 [TGG>TAG], α1 cd 59 [GGC>GAC] (Hb Adana), α2 init cd [ATG>ACG], α2 cd 19 [-G], α2 IVS1 [-5nt], α2 cd 59 [GGC>GAC], α2 cd 125 [CTG>CCG] (Hb Quong Sze), α2 cd 142 [TAA>CAA] (Hb Constant Spring), α2 cd 142 [TAA>AAA] (Hb Icaria), α2 cd 142 [TAA>TAT] (Hb Pakse), α2 cd 142 [TAA>TCA] (Hb Koya Dora), α2 poly A-1 [AATAAA-AATAAG] and α2 poly A-2 [AATAAA-AATGAA]. The blotted α-thalassemia line probe assay strip is shown in the figure 1 below.

RESULTS

The results of the molecular assay, automated haematology analyser and the HPLC for A2 have been tabulated and presented in Table No 1. The image of the line probe assay is presented as Figure 1.
Figure 1. Shows a line probe assay result for α thalassemia. The sample shows the α2 IVS 1-5 nt mutant gene to be positive.

Of the ten samples analysed nine could be successfully amplified and only one sample required retesting. One sample showed wild type α 2 Poly A missing along with the α 2 poly a-1 (AATAAA>AATAAG) mutation with a suggestive diagnosis of HbH disease. In all the other samples all the wild type genes were found positive. Five samples showed a mutation band each. A SEA double gene deletion was seen in one case suggesting α 0-thalassemia. A 3.7 single gene deletion was seen in three cases, and α 2 IVS1 (-5nt) mutation seen in one case suggesting these cases have α + thalassemia. Four samples did not show the mutation bands to be positive confirming the diagnosis of Iron deficiency anaemia.
DISCUSSION

Alpha Thalassemia is prevalent world over, with a preponderance in the tropics and sub-tropic regions. Carriers with hemoglobinopathy may have an advantage in natural selection as they are thought to be better protected from malarial parasites. Alpha thalassemia is most frequently caused by deletions involving one or more alpha globin genes and less commonly by non-deletional defects.  

PCR, GAP PCR and more recently Multiplex ligation-dependent probe amplification (MLPA) have been used to detect α thalassemia. To identify non-deletional

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<th>Bands for Wild type genes</th>
<th>Bands positive for Mutant genes</th>
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<th>Gender</th>
<th>MCV</th>
<th>RBC Count</th>
<th>RDW</th>
<th>MCH</th>
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point mutations α globin gene sequence analysis is performed\textsuperscript{4,5}. Identifying the alpha globin genotype in carriers is diagnostic and necessary for genetic counselling. Since non deletional forms are more severe than the deletional forms, patients with HbH disease require globin gene typing for prognostication. A microarray to detect the α-thalassaemia deletion and the 3.7kb and 4.2kb α+thalassaemia deletions have been described\textsuperscript{6,7}. In this study we have used a commercial line probe assay (CE approved) to detect 21 known gene mutations. The carrier rates of α thalassemia may vary from >1% to even as high as 80-90% of the population in tropical and subtropical regions of the world\textsuperscript{8}. The few published studies on α thalassemia from the region have shown that α-Thalassemia is commonly prevalent in Saudi Arabia and the United Arab Emirates. There are no large-scale study reports regarding the prevalence of α-thalassemia in the GCC and especially the UAE.

Kalla and Baysal in 1998\textsuperscript{9} and Baysal in 2011\textsuperscript{9} described studies on Cord blood samples collected from 419 consecutive new-borns of UAE nationals. Polymerase chain reaction (PCR) followed by sequencing analysis of the α-globin genes showed that 49% of the neonates had α-thalassemia. They found the incidence of the α3.7 deletion, to be extremely high. Other genotypes described were severe non-deletional type α-thalassemia such as the polyadenylation signal (polyA1) [αPA–1 (AATAAA>AATAAG)], polyA2 [αPA–2 (AATAAA>AATGAA)], Hb Constant Spring [Hb CS, α142 (αCS/αCS) TAA>CAA (α2)] and pentanucleotide deletion [α–5 nt (GAGGTGAGG>GAGG)]. The polyA1 mutation accounted for almost 50% of all the α-thal alleles, making it one of the most common mutations in the Gulf Region.

Dehbozorgian et al in 2015\textsuperscript{10} have in a large study of 3993 individuals described α-thalassaemia mutations present in Iranian population. Thirteen α-thalassemia mutations were described. Allele of α(3.7) mutation was the most prevalent (43.84%) followed by the α(IVS1/-5NT) allele with the prevalence of 4.91%.

Our pilot study also shows similar findings. A 3.7 single gene deletion was seen in three cases, and a 2 IVS1 (-5nt) mutation seen in one case suggesting these cases have α + thalassemia. One sample showed wild type a 2 Poly A missing along with the α 2 poly a-1 (AATAAA>AATGAG) mutation with a suggestive diagnosis of HbH disease. A SEA double gene deletion was seen in one case suggesting α0-thalassemia. Four samples did not show the mutation bands to be positive confirming the diagnosis of Iron deficiency anaemia. Our study shows the successful use of a simple line probe assay to detect α thalassemia. Following this preliminary study it is now planned to carry out studies encompassing a larger population.

REFERENCES