

Rapid and Cost-Effective Antenatal Diagnosis of Haemoglobin Bart's Hydrops Foetalis Syndrome Using a Duplex-Polymerase Chain Reaction

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Summary

Haemoglobin Bart's hydrops foetalis syndrome ($--^{SEA}/--^{SEA}$) is not compatible with life and contributes to a majority of the hydropic fetuses in the Malaysian Chinese α -thalassaemia carriers who possess the 2- α -gene deletion *in cis* ($--^{SEA}/\alpha\alpha$). A duplex-PCR which simultaneously amplifies a normal 136 bp sequence between the $\psi\alpha$ - α 2-globin genes and a 730 bp Southeast Asian deletion-specific sequence ($--^{SEA}$) between the $\psi\alpha$ 2- θ 1-globin genes was established. The duplex-PCR which detects the $--^{SEA}$ deletion in both chromosomes serves as a rapid and cost-effective confirmatory test in the antenatal diagnosis of Haemoglobin Bart's hydrops foetalis syndrome in Malaysia. In addition, the duplex-PCR is simple to perform as both the normal and deletion-specific α -globin gene sequences are amplified in the same PCR reaction.

Key Words: Antenatal diagnosis, Haemoglobin Bart's hydrops foetalis syndrome, Southeast Asian deletion, DNA amplification, Duplex-PCR

Introduction

Thalassaemias are one of the most common inherited single gene disorders in the world population and it is the most common inherited haematological disorder in Malaysia. The thalassaemias are a heterogeneous group of genetic blood disorders of haemoglobin synthesis, and are characterised by the absence or reduced production of one or more globin chains of haemoglobin. Normal individuals have four functional α -globin genes, α -thalassaemia usually results from the deletion of 1, 2, 3 or 4 of the α -globin genes and this causes a progressive decrease in α -globin chain synthesis in carriers of α -thalassaemia^{1,2}. The clinical phenotypes of α -thalassaemia carriers vary according to the number of α -globin genes deleted³. Carriers with three α -globin genes ($-\alpha/\alpha\alpha$) and two α -globin genes ($--/\alpha\alpha$ or $-\alpha/-\alpha$) have α -thalassaemia trait. Carriers with

only one functioning α -globin gene ($--/\alpha$) show Haemoglobin H (β_4 tetramers) disease, which is characterised with marked unbalanced globin-chain synthesis ratio⁴. Inheritance of no functional α -globin genes ($--/--$) is incompatible with life and leads to Haemoglobin Bart's (γ^4 tetramer) hydrops foetalis syndrome^{5,6,7}.

Hb Bart's hydrops foetalis syndrome in Southeast Asia is commonly caused by a 20.5 kilobases Southeast Asian deletion ($--^{SEA}$)⁸. The deletion removes the $\psi\alpha$ 2, $\psi\alpha$ 1, α 2, α 1 and θ -globin genes and leaves only the ζ -genes (ζ 2 and $\phi\zeta$ 1) intact⁹. Infants with Hb Bart's hydrops foetalis syndrome following a gestation of 23–43 weeks usually die *in utero*, during delivery or within six hours of birth¹⁰. Apart from foetal death, Hb Bart's hydrops foetalis syndrome is also characterised by a high incidence of serious maternal complications in pregnancies¹¹.

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The single α -globin gene deletions are the most common forms of α -thalassaemia, with the 2 major types, $-\alpha^{37}$ and $-\alpha^{42}$. The prevalence of the single α -globin gene deletion is common especially in populations in the Malaria belt and its frequency can be as high as 80% in some areas in India, Nepal and the Southwest Pacific¹². Deletions of both α -globin genes within the same chromosome and in addition, the non-deletional forms of α -thalassaemia, are less common and reported particularly in Southeast Asia and in the Mediterranean¹³.

There is a 25% risk of a pregnancy with Hb Bart's hydrops foetalis in couples who are both α -thalassaemia carriers ($--/\alpha\alpha$). The identification of α -thalassaemia is thus crucial, since antenatal diagnosis and genetic counselling can be offered to families at risk for a Hb Bart's hydrops foetalis pregnancy. Antenatal diagnosis for Hb Bart's hydrops foetalis syndrome caused by the $--^{SEA}$ deletion can be carried out by gap-PCR across the α -globin gene complex.

Antenatal diagnosis for α -thalassaemia was established in the Department of Molecular Medicine, University of Malaya since 1996¹⁴. Molecular characterisation of α -thalassaemia is currently carried out using two separate DNA amplification protocols – one for amplification of a normal sequence between the $\psi\alpha$ - $\alpha 2$ -globin genes, and the other for the specific amplification of the Southeast Asian deletion ($--^{SEA}$). The Southeast Asian (SEA) deletion is responsible for the majority of α -thalassaemia in the Malaysian Chinese and thus contributes to almost a 100% of Hb Bart's hydrops foetalis syndrome in this ethnic group¹⁵. DNA amplification using the 2 separate DNA amplification protocols is sensitive and specific, and produces distinct PCR products. However, the absence of amplification products for the normal $\psi\alpha$ - $\alpha 2$ -globin gene sequence in DNA from Hb Bart's hydrops foetalis can represent false negative results due to sub-optimal PCR amplification conditions. Thus, separate internal controls to amplify a specific region of the β -globin gene are used to confirm optimal PCR conditions in DNA from Hb Bart's hydrops foetalis cases. In addition, antenatal diagnosis using 2 separate DNA amplification protocols can be time consuming as the tests are carried out sequentially unless two thermal cyclers are available in the laboratory. Furthermore, the 2 separate DNA amplification protocols require about twice the amount of DNA compared with antenatal diagnosis using the duplex-PCR.

This study reports on the development and evaluation of a duplex-PCR that simultaneously amplifies both the

normal sequence between the $\psi\alpha$ - $\alpha 2$ -globin genes and the SEA-specific deletion in a single PCR reaction. The duplex-PCR offers a rapid and more cost-effective antenatal diagnosis method for α -thalassaemia in the Malaysian population.

Materials and Methods

Background

Antenatal diagnosis for α -thalassaemia in the Department of Molecular Medicine, University of Malaya, is currently carried out using two separate DNA amplification protocols – one for amplification of a normal sequence between the $\psi\alpha$ - $\alpha 2$ -globin genes, and the other for the specific amplification of the Southeast Asian deletion ($--^{SEA}$). Ethical and Institutional Approval was obtained for this study from the Ethics Committee of the University Malaya Medical Centre in accordance to the Declaration of Helsinki. In addition, informed and signed consent were obtained from the couple before any antenatal diagnosis was carried out. Sampling procedures and outcomes of the tests were explained to the couple by a qualified consultant, the gynaecologist in charge of the pregnancy. Thus, DNA testing was always preceded by appropriate counselling, and post-counselling was given during discussion of the antenatal results.

A total of 145 antenatal diagnoses cases already confirmed for their α -thalassaemia genotypes using the two DNA amplification protocols were randomly picked for development and evaluation of the duplex-PCR. The cases comprised of 19 foetal blood, 126 chorionic villi and 290 parental blood samples. These antenatal diagnoses cases were requested from 14 clinics/hospitals in Malaysia – University Malaya Medical Centre, Kuala Lumpur General Hospital, Foetal Medicine and Gynaecology Centre, Subang Jaya Medical Centre, Subang Women's Clinic, Sunway Medical Centre, Gleneagles Intan Medical Centre, Damansara Specialist Hospital; Seremban Hospital, Negeri Sembilan; Ipoh Hospital, Klinik Pakar Wanita K.C. Foo, Ipoh; Penang Hospital, Island Hospital and Loh Guan Lye Specialists Centre, Penang.

Chorionic villi sampling and DNA preparation

Chorionic villi (CV) samples (n =126) were obtained at around 12-14 weeks of gestation by the transabdominal approach under ultrasound guidance. The CV samples were examined using a zoom stereo microscope to exclude maternal contamination. The CV were washed several times in physiological saline and digested in

solution containing 10 mmol/L Tris-HCl, 1 mmol/L EDTA, 0.15 mol/L NaCl, 10% sodium dodecyl sulphate and 30-50 μ L of 10 mg/mL proteinase K at 37°C overnight. DNA was purified by phenol-chloroform and then precipitated in 3 mol/L sodium acetate and 2 volumes ethanol. Aqueous DNA was washed in alcohol, dried and then solubilised in double distilled water. DNA concentration and purity were measured spectrophotometrically.

DNA preparation of adult and foetal blood sampling

Foetal blood samples (n =19) were obtained from foetuses between 18-34 weeks of gestation by cordocentesis under ultrasound guidance. Foetal blood samples were stained using the Kleihauer test to check for maternal contamination and also stained with the standardized Romanowsky stain to check for nucleated red blood cells¹⁶. Whole blood samples were collected into sterile K₂EDTA blood collection tubes from the 145 couples (n =290) who requested antenatal diagnosis. DNA was extracted using proteinase K and sodium dodecyl sulphate. Extracted DNA was purified by phenol-chloroform and precipitated with ethanol.

DNA amplification

The 136 bp normal sequence between the $\psi\alpha$ - α 2-globin gene region and the 730 bp --^{SEA} deletion-specific sequence are currently amplified separately in our laboratory in two single-PCR reactions.

Amplification of the 730 bp --^{SEA} deletion-specific sequence

The --^{SEA} deletion-specific sequence was amplified using primers flanking the $\psi\alpha$ 2- θ 1-globin genes along the α -globin gene complex. Amplification of the 730 bp sequence was performed using a pair of oligonucleotide primers, SEA1: 5'-CTCTGTGTTCTCAGTATTGGAGG-3' and SEA2: 5'-ATATATGGGTCTGGAAGTGTATC-3'¹⁷ in 200 μ M of each deoxynucleotide triphosphate (dNTP), 2.5 mM magnesium chloride (MgCl₂), 2.5 units of *Taq* DNA polymerase and 1 μ g DNA. DNA amplification using 20 pmol of each primer was carried out for 30 cycles at 93°C for 1 minute (min), 60°C for 1 min and 72°C for 1.5 min.

Amplification of the 136 bp $\psi\alpha$ - α 2-globin gene sequence

The 136 bp normal sequence between the $\psi\alpha$ - α 2-globin genes was amplified using the primers α 1: 5'-TACTGTAGATACCCGTGTACAA-3' and α 2: 5'-ATCATGATGGAAACATAGTAAT-3' as described by Chehab *et al.*, 1987¹⁸. The in-house modified DNA amplification was performed using 2.5 units of *Taq* DNA polymerase, 15 pmol of each primer, 200 μ M of

each dNTP, 1.5 mM MgCl₂ and 1 μ g DNA. The PCR mixture was amplified for 30 cycles with denaturation at 93°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 63°C for 45 seconds.

The duplex-polymerase chain reaction

The duplex-PCR was performed by combining 20 pmols of primers SEA1 and SEA2, 25 pmols of primers α 1 and α 2, with 1 μ g DNA, 2.5 units of *Taq* DNA polymerase and 200 μ M of each dNTP. Magnesium chloride (MgCl₂) was evaluated at different concentrations for optimal DNA amplification of the two DNA sequences. The optimal MgCl₂ used in the duplex-PCR was determined at 1 mM. The PCR cycling conditions were initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 93°C for 1 min, annealing at 50°C for 1 min, synthesis at 72°C for 3 min and a final extension at 72°C for 6 min. DNA from a known heterozygote for α -thalassaemia ($\text{--}^{\text{SEA}}/\alpha\alpha$), a homozygous α -thalassaemia ($\text{--}^{\text{SEA}}/\text{--}^{\text{SEA}}$) and a normal individual ($\alpha\alpha/\alpha\alpha$) were amplified concurrently in every PCR to act as positive and negative controls.

DNA analysis using the Amplification Refractory Mutation System (ARMS)

The β -mutations at CD41/42 (-CTTT) and -28 (A-G) were detected using the Amplification Refractory Mutation System (ARMS). The primers used for specific amplification of the CD41/42 mutation were 5'-GAGTGGACAGATCCCCAAAGGACTCAACCT-3' and 5'-ACCTCACCTGTGGAGCCAC-3'. Amplification of the -28 β -globin gene mutation was performed using primers 5'-AGGGAGGGCAGGAGCCAGGGCTGGGCTTAG-3' and 5'-CCCCTTCCTATGACATGAACCTAA-3'. The ARMS was carried out using 20 pmol of each primer, 200 mM of each dNTP, 2.5 mM MgCl₂, 1 unit of *Taq* DNA polymerase and 1 μ g DNA. Amplification was carried out with an initial denaturation at 95°C for 5 min followed by 30 cycles at 93°C for 1 min, 65°C for 1 min, 72°C for 1.5 min. A final extension was carried out at 72°C for 3 min. The ARMS produced a specific 443 bp CD41/42 and a 624 bp -28 amplified product.

Gel electrophoresis of PCR products

Amplified PCR products were resolved by electrophoresis on 1.5% agarose gels. Electrophoresis was carried out at 90 V for 45 minutes. DNA bands were observed using ultraviolet light irradiation after ethidium bromide staining.

Results

The 145 antenatal cases consisted of blood from both husband and wife. Each antenatal case also involved

either a foetal blood sample or chorionic villi sample. A total of 19 foetal blood and 126 CV samples were analysed. DNA was extracted and genotyped using the two separate DNA amplification protocols – the 136 bp $\psi\alpha$ - α 2-globin gene sequence and the 730 bp --SEA deletion-specific sequence. All the DNA samples were also amplified using the duplex-PCR to determine its sensitivity and specificity for antenatal diagnosis of α -thalassaemia. The results from both PCR protocols – separate DNA amplification of the normal and deletion-specific α -globin gene sequences and the duplex-PCR – were compared to determine the correlation between the two protocols.

Amplification of the 730 bp --SEA deletion-specific sequence and 136 bp $\psi\alpha$ - α 2-globin gene sequence in 2 separate DNA amplification protocols

Figure 1 shows the electrophoresis of PCR products of an antenatal diagnosis case after amplification of the normal 136 bp sequence between the $\psi\alpha$ - α 2-globin genes and amplification of the 730 bp Southeast Asian deletion (--SEA) sequence between the $\psi\alpha$ - θ 1-globin genes. The parents are α -thalassaemia carriers with the SEA deletion ($\text{--SEA}/\alpha\alpha$). Their DNA has amplified both the 730 bp SEA deletion-specific sequence and normal 136 bp $\psi\alpha$ - α 2-globin gene sequence (lanes 3 & 5, upper and lower gels). The foetal DNA has only amplified the SEA deletion-specific sequence (lane 2, upper gel). The 136 bp $\psi\alpha$ - α 2-globin gene sequence in the foetal DNA was not present (lane 2, lower gel), thus, indicating the foetus as a Bart's hydrops foetalis ($\text{--SEA}/\text{--SEA}$). Lane 4 contains DNA from a normal individual ($\alpha\alpha/\alpha\alpha$) and showed amplification of only the 136 bp $\psi\alpha$ - α 2-globin gene sequence. Lane 6 contains DNA from an α -thalassaemia carrier ($\text{--SEA}/\alpha\alpha$), and thus has amplified both the 2 sequences. Lane 7 in the upper and lower gels are the negative controls for amplification of the SEA deletion-specific sequence (DNA from aa/aa individual) and the 136 bp $\psi\alpha$ - α 2-globin gene sequence (DNA from $\text{--SEA}/\text{--SEA}$ foetus) respectively, thus, no PCR products are present in both gels. Lane 8 is the DNA blank where no DNA was added to the PCR reaction.

Duplex-polymerase chain reaction

In the duplex-PCR, detection of the --SEA deletion-specific sequence and the normal $\psi\alpha$ - α 2-globin gene sequence was carried out simultaneously in a single PCR reaction (Figure 2). DNA from a couple with α -thalassaemia amplified both the 730 bp SEA deletion-specific sequence and normal 136 bp $\psi\alpha$ - α 2-globin gene sequence (lanes 3 & 6) confirming their α -thalassaemia carrier status. The foetal DNA has only amplified the

SEA deletion-specific sequence and the 136 bp $\psi\alpha$ - α 2-globin gene sequence was not present (lane 4), thus, indicating the foetus as a Bart's hydrops foetalis ($\text{--SEA}/\text{--SEA}$). Controls for the duplex-PCR include DNA from normal individuals ($\alpha\alpha/\alpha\alpha$, lanes 2 & 5), α -thalassaemia carrier ($\text{--SEA}/\alpha\alpha$, lane 7) and from a Bart's hydrops foetalis ($\text{--SEA}/\text{--SEA}$, lane 8). Lane 9 is the DNA blank.

Detection of α -thalassaemia

DNA from the 145 antenatal diagnosis cases was analysed using the 2 separate DNA amplification protocols and duplex-PCR. In the 126 antenatal cases using CV, all the couples were confirmed as α -thalassaemia carriers. In addition, 2 fathers were further found to have $\alpha\beta$ -thalassaemia – one with the β -mutation at CD41/42 and the other at -28. One mother was also confirmed as an $\alpha\beta$ -thalassaemia carrier with the CD41/42 mutation. Another mother was detected with Hb H disease (genotype $\text{--SEA}/\alpha^{1,2}$). Antenatal diagnosis showed 26.2% pregnancies with Hb Bart's hydrops foetalis syndrome, 47.6% fetuses were determined as α -thalassaemia carriers with the Southeast Asian deletion, 24.6% fetuses were normal, 0.8% were diagnosed with $\alpha\beta$ -thalassaemia and 0.8% with Hb H disease (Table I).

In the 19 pregnancies using foetal blood for antenatal diagnosis, 18 of the fetuses already showed hydropic features during ultrasound scanning (soft tissue oedema, pericardial effusions, pleural effusions and ascites). An increased number of nucleated red blood cells in the foetal blood film was observed after Romanowsky staining. In three pregnancies, the parents and fetuses showed absence of the --SEA deletion and presence of the normal sequence between the $\psi\alpha$ - α 2-globin genes indicating that the hydropic fetuses were not $\text{--SEA}/\text{--SEA}$. For the remaining 16 cases, all the couples were found to be α -thalassaemia carriers ($\text{--SEA}/\alpha\alpha$) with exception to one mother with Hb H disease ($\text{--SEA}/\alpha^{3,7}$). Ninety four per cent of the pregnancies were confirmed with Hb Bart's hydrops foetalis syndrome and one foetus with Hb H disease ($\text{--SEA}/\alpha^{3,7}$) (Table I). The percentage of pregnancies with Hb Bart's hydrops foetalis syndrome was high in the foetal blood samples and this can be explained by the fact that 95% of these pregnancies already showed hydropic features when registered for antenatal diagnosis for α -thalassaemia.

The results obtained using the duplex PCR showed a 100% correlation with results obtained using the 2 separate DNA amplification protocols. There was no discrepancy in any of the results obtained.

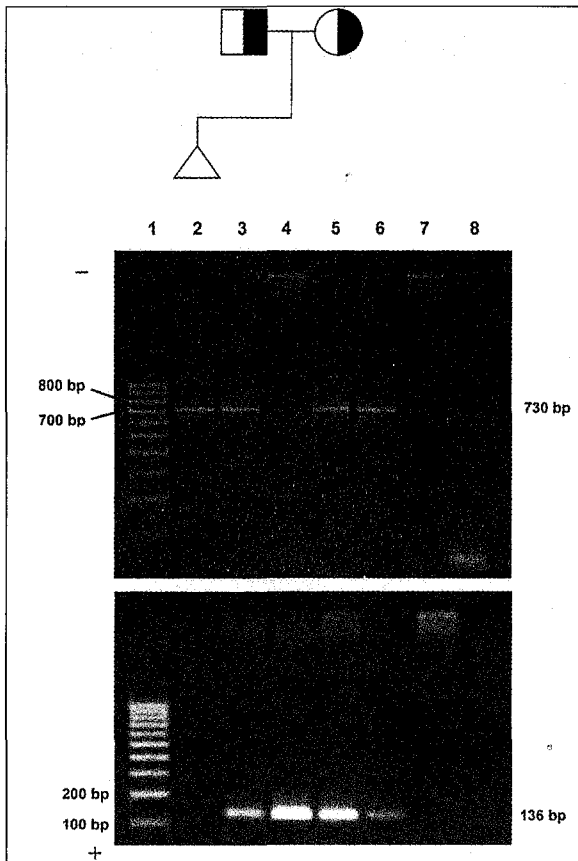


Fig. 1:

Agarose gel electrophoresis of the amplified $--_{SEA}$ deletion-specific sequence (upper gel) and the normal sequence between the $\psi\alpha$ - α 2-globin gene (lower gel). Lane 1: 100 bp DNA ladder. Lane 2: DNA from a foetus with Hb Bart's hydrops foetalis syndrome ($--_{SEA}/--_{SEA}$), 730 bp band amplified, 136 bp band not present. Lane 3: DNA from father with α -thalassaemia ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 4: Control DNA from normal individual ($\alpha\alpha/\alpha\alpha$), 136 bp band amplified, 730 bp band not present. Lane 5: DNA from mother with α -thalassaemia ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 6: Control DNA from α -thalassaemia carrier ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 7: Negative controls ($\alpha\alpha/\alpha\alpha$ for upper gel and $--_{SEA}/--_{SEA}$ for lower gel). Lane 8: DNA blank (no DNA added). Lane 9: DNA blank.

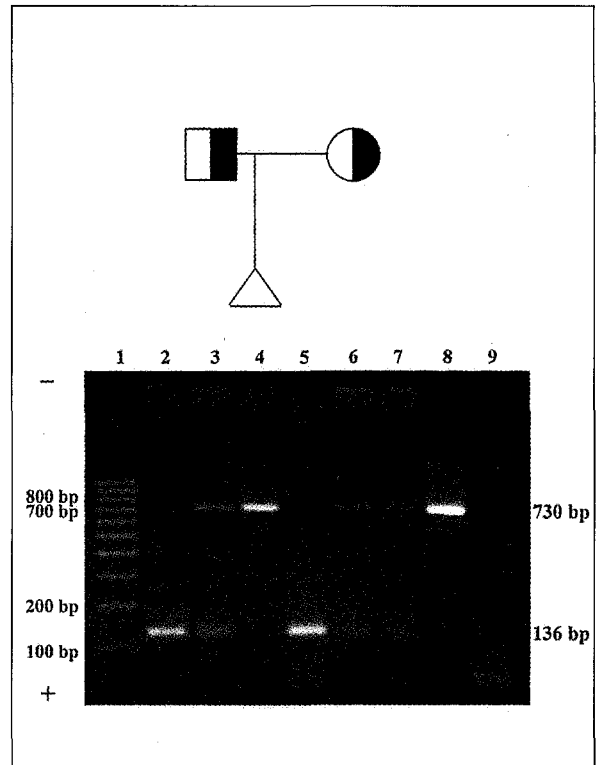


Fig. 2:

Agarose gel electrophoresis of the amplified $--_{SEA}$ deletion-specific sequence and the normal sequence between the $\psi\alpha$ - α 2-globin gene in a duplex-PCR. Lane 1: 100 bp DNA ladder. Lane 2: Control DNA from normal individual ($\alpha\alpha/\alpha\alpha$), 136 bp band amplified, 730 bp band not present. Lane 3: DNA from father with α -thalassaemia ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 4: DNA from a foetus with Hb Bart's hydrops foetalis syndrome ($--_{SEA}/--_{SEA}$), 730 bp band amplified, 136 bp band not present. Lane 5: Control DNA from normal individual ($\alpha\alpha/\alpha\alpha$), 136 bp band amplified, 730 bp band not present. Lane 6: DNA from mother with α -thalassaemia ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 7: Controls DNA from α -thalassaemia carrier ($--_{SEA}/\alpha\alpha$), 730 bp and 136 bp bands amplified. Lane 8: Control DNA from Hb Bart's hydrops foetalis ($--_{SEA}/--_{SEA}$), 730 bp band amplified, 136 bp band not present. Lane 9: DNA blank.

Discussion

In the Malaysian Chinese, 4.5% are carriers of the α -thalassaemia 1 or α^0 -thalassaemia gene ($-\text{SEA}/\alpha\alpha$) and thus are at risk of having a foetus with Hb Bart's hydrops foetalis syndrome¹⁵. On the other hand, the Malaysian Malay α -thalassaemia carriers more frequently possess the single α -globin gene deletion and therefore are seldom at risk for a Hb Bart's hydrops foetalis pregnancy. In the Malays who have a deletion of two α -globin genes, the presence of the gene deletions are more commonly *in trans* ($-\alpha/-\alpha$).

The duplex-PCR utilised the same four primers as in the two separate DNA amplification protocols, thus, new primer sequences were not purchased. In the development of the duplex-PCR, optimisation was carried out using different primer and MgCl₂ concentrations to ensure optimal amplification of both the 730 bp and 136 bp bands. In addition, different annealing temperatures were evaluated to ensure optimal binding of the four different primers to their complementary sequences in the template DNA. This was very necessary as the primers had different melting points. The *Taq* DNA polymerase concentration for the duplex-PCR was used at 2.5 units per reaction.

Detection of α -thalassaemia by DNA amplification of both the $-\text{SEA}$ deletion-specific sequence and the normal sequence between the $\psi\alpha$ - α 2-globin genes in a duplex-PCR allows a more rapid and cost-effective analysis of DNA from adult blood, foetal blood and chorionic villi samples. The cost of the diagnosis is reduced to nearly 50% using the duplex-PCR as additional internal controls are not necessary, and positive and negative controls need only be amplified once. The workload and time spent carrying out the molecular study is also reduced with the use of the duplex-PCR.

Technical variation due to inter-laboratory variability is common, thus, appropriate controls should be included in every PCR reaction. Results which rely on the absence of an amplified band (as in the 2 separate DNA amplification protocols) may have resulted from sub-optimal PCR conditions. In the duplex-PCR, DNA from Hb Bart's hydrops foetalis which does not amplify the 136 bp normal sequence amplifies the 730 bp $-\text{SEA}$ deletion-specific sequence in the same PCR reaction tube (Figure 2, lanes 4 & 8). Similarly, DNA from normal individuals which do not amplify the 730 bp $-\text{SEA}$ deletion-specific sequence will amplify the 136 bp

normal sequence (Figure 2, lanes 2 & 5). Thus, there is little ambiguity as to whether optimal PCR conditions are present during DNA amplification.

A number of protocols have recently been developed for molecular characterisation of α -thalassaemia. The highly sensitive and specific developed protocols include reverse dot-blot assays¹⁹, multiplex-ARMS²⁰ and multiplex-PCR^{21,22}. The reverse dot-blot and Multiplex-ARMS methods have been developed for detection of non-deletional α -thalassaemia^{19,20}, while the multiplex-PCRs determine deletional and α -globin gene triplication^{21,22}. In Malaysia, the common defect responsible for α -thalassaemia involves the deletion of one or both α -globin genes. In addition, Hb Bart's hydrops foetalis syndrome observed in the majority of hydropic pregnancies in the Chinese results from the inheritance of the double α -gene deletion from both parents. Thus, a simple molecular test for the confirmation of the double α -globin gene deletion in both chromosomes is sufficient for the antenatal diagnosis of Hb Bart's hydrops foetalis syndrome in Malaysia. The duplex-PCR developed and established in this study is simple to perform and does not require staff to be highly trained in molecular genetics. The duplex-PCR also requires only one thermal cycler and this is generally affordable in general hospitals and small institutions or centres carrying out molecular work. Since the duplex-PCR amplifies both a normal 136 bp sequence between the $\psi\alpha$ - α 2-globin genes and the $-\text{SEA}$ deletion-specific sequence in a single PCR reaction, less DNA is required for molecular analysis. This is especially important, as only minute quantities of CV and foetal blood are available for genetic testing during antenatal diagnosis. The optimisation of the PCR conditions and the minimum use of primers, positive and negative controls and *Taq* DNA polymerase for the duplex-PCR allow it to be rapid and cost-effective.

Conclusion

In conclusion, the duplex-PCR which determines both the 136 bp normal sequence between the $\psi\alpha$ - α 2-globin genes and the 730 bp Southeast Asian deletion sequence between the $\psi\alpha$ 2- θ 1-globin genes in a single PCR reaction is developed as a rapid and cost-effective test for antenatal diagnosis of Hb Bart's hydrops foetalis syndrome in Malaysia.

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