CHAPTER IV

Discussion and Conclusion

α-thalassemia 1 is one of the most common form of thalassemia in Thailand (Wasi *et al.*, 1980). The most common α-thalassemia 1 occurs from a deletion of about 20 kb that called α-thalassemia 1 Southeat Asia type (Fucharoen *et al.*, 1994). But, some cases of non-deletion α-thalassemia show the phenotype expression same with α-thalassemia trait such as the homozygous for Hb Constant Spring would be anticipated to have a hematologic abnormality similar to that of α-thalassemia trait (Honig and Adams, 1986).

Prevention and control of thalassemia in the entire population requires a well planed program to establish the epidemiology of the disorders and education to rise the awareness of genetic risk in the medical profession and the population at large. Accurate diagnosis and advice should be provide to the carrier, the best possible management should be made available to the affected patients. Prevention of further thalassemia offspring in the case of couples at risk can be planned by prenatal diagnosis. This approach is cost-effective, and is proving remarkably successful in reducing the frequency of thalassemia in Mediterranean countries (Kuliev 1988, Loukopoulos *et al.*,1988). In Thailand, it has been estimated that the total cost for treating major thalassemia disease is about (US$)220 million per year (Parnsatienkul,

1990). But elsewhere the cost of a total prevention program has been demonstrated to be 1/5th to 1/10th of the cost of treating the existing affected patients (Modell *et al.*, 1984).

Couples at risk are usually identified by a prior α-thalassemia hydrops pregnancy,. but the screening for couples with α-thalassemia 1 SEA-type can also be effective. Prenatal diagnosis of α-thalassemia may be performed by restriction mapping obtained during the first trimester of gestation (Goosens *et al.*, 1983) and the use of radioactivity labeled α and ζ human globin probe which the ζ genes remain intact and are located on unique restriction fragment (Pressley *et al.*, 1980). However, these technique are expensive, time-consuming and require a large amount of genomic DNA (>3μg). More rapid and sensitive approaches to DNA diagnosis utilizing slot blot, allele specific oligonucleotide probe and polymerase chain reaction are rapidly being developed and adapted to α-thalassemia 1. These more recent approaches significantly decrease the amount of material needed for analysis and increase the speed of the diagnosis procedure. In this study, the potocol of Kitsirisakul et al. (1996) using the primers described by Chang et al. (1991) is the method for the diagnosis of α-thalassemia 1 SEA-type in pregnant women who come for ANC at Maharaj Nakorn Chiang Mai hospital. The PCR primers A and C located far apart in normal DNA will not direct amplification unless a deletion bring them into closer proximity which

gives a specific band of 194 bp. Whereas primers A and B located close in normal DNA will direct specific band at 314 bp in normal sequence. Thus, human who carries normal sequence will have only specific band at 314 bp whereas the carrier will have specific band at 194 bp and 314 bp. These PCR method can be applies for prenatal diagnosis of Hb Bart's hydrops fetalis which is caused by homozygous α-thalassemia 1. In this case only a specific band of 194 bp is detected.

Five hundred pregnant women were screened in this study which the example shown on figure 11. The lanes that show a specific band of 194 bp are α-thalassemia 1 of SEA type carriers. The lane that show no band are not α-thalassemia 1 of SEA traits. But the possibility of false negative PCR results has to be taken into account. False negative results could be obtained when blood that is too old or blood that was not stored properly is received for analysis. On the other hand if template DNA or any of the PCR components like dNTPs, *Taq* DNA polymerase, primers etc. was forgotten to be added, PCR can not be successful too. The latter risk is minimized when all the PCR components are combined in a mastermix and an aliquot of this mastermix is added to the DNA templates, like in this work. If it would be possible to combine all three primers in a single PCR and using a mastermix the risk of false negative results would be virtually eliminated since all samples should show at least the 314 bp band from normal DNA. The α-thalassemia 1 of SEA

type deletion was found in 44 women. The percentage of carrier is 8.8% (44/500×100), according to Hardy-Weinberg equilibrium 42 carriers of α-thalassemia 1 of the SEA-type are expected. The calculated frequency of α-thalassemia 1 of the SEA-type is 0.044 (44/1000), and the expected frequency of homozygous α-thalassemia 1 of the SEA-type, the cause of fetal Hb Bart's hydrops fetalis, is therefore 0.00194 (0.044×0.044) (Kitsirisakul *et al.*, 1996). Thus, approximately 2 Hb Bart's hydrops fetalis cases can be predicted in 1000 births. This study demonstrates that the PCR technique is very helpful in detecting carriers of α-thalassemia 1 of the SEA-type, the first step of the prenatal diagnosis of Hb Bart's hydrops fetalis. Moreover, the PCR technique is rapid since DNA extraction, in *vitro* amplification and subsequent analysis can be done in one working day. Although PCR is a rapid and specific method to detect α-thalassemia 1 but it is not available in most community hospital. Thus, to develop a simple screening test for the detection of thalassemia carrier is necessary. The technique should be simple enough to be suitable for use at the small district hospital level.

In the present study, the osmotic fragility test and HbA_2 determination were used to screen for α-thalassemia 1 traits. The osmotic fragility test involves the resistance of thalassemic red cell in hypotonic solution. The one step glycerol-saline test, a rapid, simple

and reliable method for determination of the red cell osmotic fragility was proposed in 1980 by Flatz and Flatz as a useful mass screening procedure for β-thalassemia. Whereas, HbA_2 which is a minor component of normal adult Hb were determined by microcolumn ion-exchange chromatography. This method involve the ionic strength of each type of hemoglobins. This method is rapidly and accurately (Efremov *et al.*, 1974). The value of HbA_2 in α-thalassemia 1 is lower than 4% (Fuchroen *et al.*, 1988, Tocharus *et al.*, 1995, Sanguansermsri 1992). But, 4 -10% HbA_2 value present as β-thalassemia trait and HbA_2 higher than 10% present as Hb E.

The determination of HbA_2 in blood of 500 pregnant women by microcolumn chromatography were shown 67 cases are abnormal which may be HbE or β-thalassemia trait. Whereas the osmotic fragility test were shown 135 case estimate as abnormal. But only 44 cases carries α-thalassemia 1 traits. 99 cases of no α-thalassemia 1 traits may by present as HbE, β-thalassemia trait or iron deficiency. The precision of these two methods were in accept range which had %CV less than 10%. The α-thalassemia 1 carrier which detected by PCR have correlation with osmotic fragility test at HbA_2 lower than 4%. The α-thalassemia 1 carrier had osmotic fragility test value less than 56% hemolysis. There was significant difference of osmotic fragility between normal and α-thalassemia 1 carrier that p value was 0.0001. The mean and standard

deviation of normal are 74.55±20.9 whereas the carriers are 32.82±12.45. This study demonstrated that the osmotic fragility test useful as a mass screening test of α-thalassemia 1 in small hospital, although the accuracy less than PCR. These two methods are simple, well quality control and rapid which about 20-30 cases can applied in one day.

In conclusion, the hot start PCR method in this study is too difficult because much more step in the reaction. There are two reactions per sample (Primer A/C and A/B) which is time consuming. In a further study, all three primers should be combined together (PrimerA/B/C) in one reaction to avoid false negative PCR results and increase speed, lower the cost of PCR by 50%. Primer C should be designed no self homology to perform conventional PCR instead of the complicated hot start PCR. Whereas EOFT helps to decrease the workload of PCRs by more than 70%. This technique is more easier than PCR and more cheaper on routine basis. So, the osmotic fragility test is appropriate techniques for a mass pre-screening for α-thalassemia 1 SEA-type in rural area and small hospital.