CHAPTER III

RESULT

3.1 Hb A_2 Determination

The Hb A_2 levels of the 500 pregnant women were analyzed by microcolumn chromatography shown in Table 4. There are 433 blood samples have 1-4% Hb A_2. 1-4% Hb A_2 present as normal or α-thalassemia 1 carrier that reported in previous study (Sanguansermsri, 1994). Whereas, there are 35 blood samples have 4.1-10% Hb A_2 which present as β-thalassemia carrier (Marengo-Rowe *et al.*, 1965) and 32 blood samples have %Hb A_2 higher than 10% which present as Hb E (Effremov *et al.*,1974).

Figure 7 show quality control of Hb A_2 determination. The precision of this method has shown coefficient of variation (%CV) in acceptable value. The %CV was 9.408.

Table 4 Hb A_2 levels of the 500 analyzed pregnant women

% Hb A_2	No. of sample
1- 4	433
4.1-10	35
higher than 10	32

Hemolysate from the 500 pregnant women were analyzed by DEAE-Sephadex microcolumn chromatograhpy as described in material and methods.

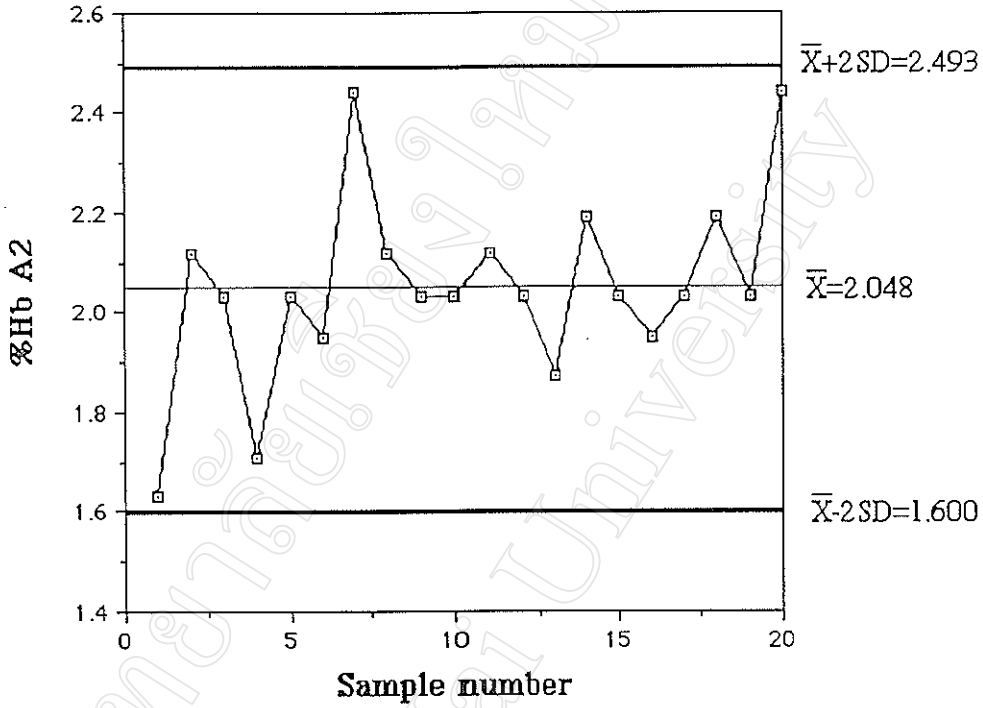

Figure 7. The quality control of HbA_2 determination

The hemolysate of the third sample of the pregnant women was run 20 times in microcolumn chromatography for detect precision of the method. Mean (X), standard deviation (SD) and coefficient of varieation were analysed by criketgraph 1.3.2 programe. Mean of Hb A_2 was 2.048 and standard deviation was ± 0.193.

3.2 Erythrocyte osmotic fragility test

The hemolysate measument of red blood cell of the 500 pregnant women were analyzed by erythrocyte osmotic fragility test devided into two cases. 0-60% EOFT which present as abnormal that reported in previous study (Flatz and Flatz 1980) have 135 cases whereas %EOFT higher than 60% which present as normal have 365 cases. The kinetic of hemolysis of red blood cells between normal person and carrier shown in figure 8.

In figure 9 show quality control of osmotic fragility test. The precision of this method has shown coefficient of variation (%CV) in acceptable value. %CV was 0.634.

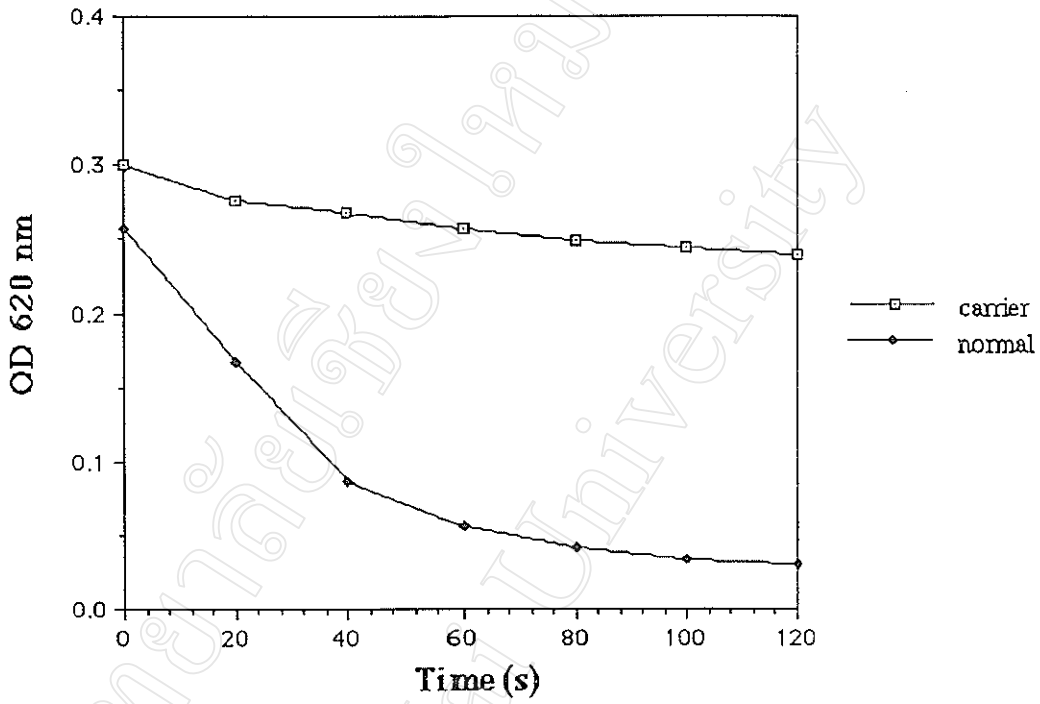

Figure 8. The kinetic of hemolysis of normal person and α-thalassemia 1 carrier

Definitions :

Normal person : α-thalassemia 1 is not detected by PCR, EOFT value higher than 60%. α-thalassemia 1 carrier : α-thalassemia 1 chromosome detected by PCR, EOFT value lower than 60%.

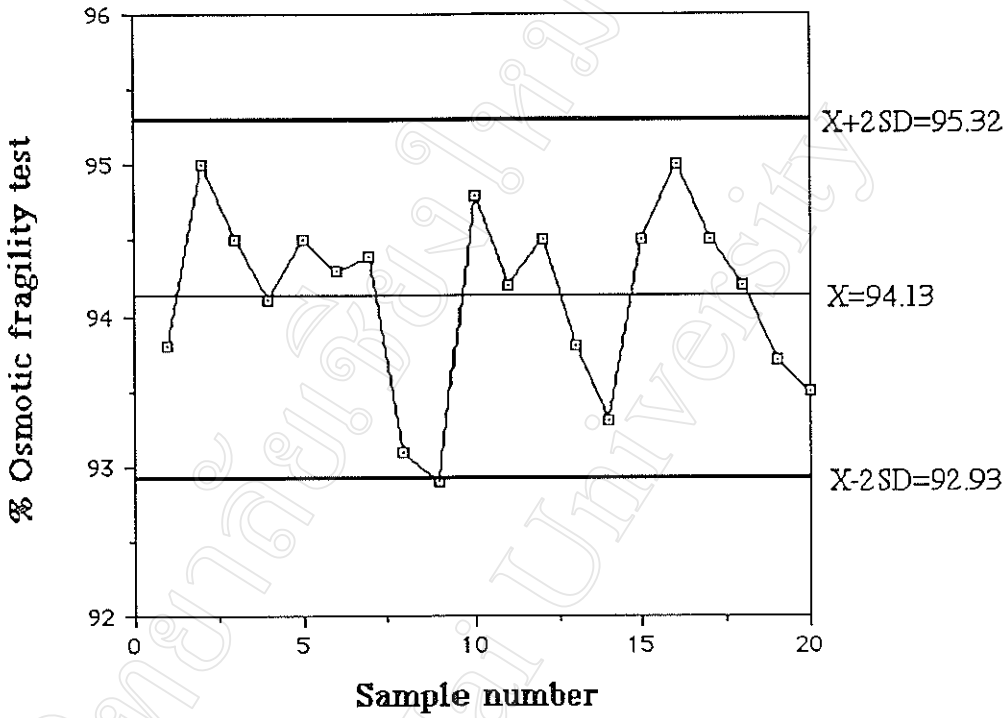

Figure 9. The quality control of erythrocyte osmotic fragility test

The hemolysate of the third sample of the pregnant women was run 20 times for detect precision of the method. Mean (X), standard deviation (SD) and coefficient of variation were analysed by cricketgraph 1.3.2 programe. Mean of erythrocyte osmotic fragility was 94.13 and standard deviation was ± 0.596.

3.3 Polymerase Chain Reaction

The PCR components and PCR condition had been optimized to provide a clear single band at the expected size of 194 bp and 314 bp as shown in figure 10. The specific band at 194 bp is the PCR product of primer A and C which used for detect α-thalassemia 1 SEA-type trait. The specific band at 314 bp is the PCR product of primer A and B which is used for detection of the normal sequence. The size of the specific bands is like expected.

Amplification of the DNA from the first 10 women to detected α-thalassemia1 of the SEA-type is shown in figure 11. An α-thalassemia1 carrier and no template were use as positive control and negative control respectively. Five hundred pregnant women were screened and the α-thalassemia 1 SEA-type was found in 44 women.

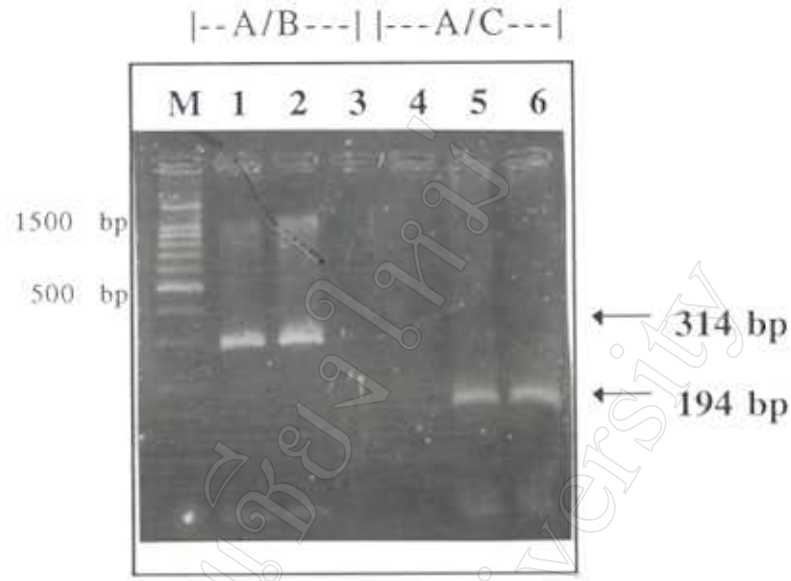

Figure 10. Detection of α-thalassemia-1 SEA type by PCR

Lane M = molecular weight marker (100 bp DNA ladder from Promega, Cat. No.G-2101) the marker size of 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, respectively, Lane 1,4 = normal sample, Lane 2,5 = α-thalassemia 1 carrier sample, Lane 3,6 = Hb Bart's hydrops fetalis sample. PCR products of primer A and B are shown in lane "1-3" whereas in lane "4-6" are shown the PCR products of primer A and C. Blood samples which collected from normal sample and α-thalassemia1 carrier were extracted and applied for PCR following the method in chapter II. The PCRs result were analyzed by agarose gel electrophoresis and visualised by ethidium bromide on UV-transilluminator. Whereas amniotic fluid (Hb Bart's hydrops fetalis sample) was extracted by chelex method (Walsh *et al.*, 1991).

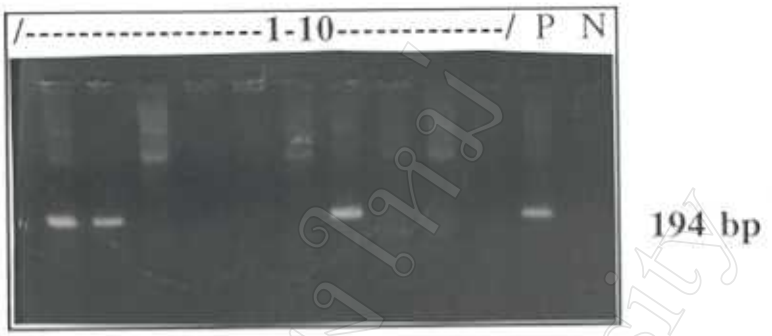

Figure 11. Amplification of the DNA from the first 10 women using primer A and C

P= positive control (Hb Bart's hydrops fetalis or α-thalassemia 1 carrier). **N** = negative control (no template in PCR). The first 10 women are shown as examples of PCR products detected by primer A/C. The DNA was extracted form blood and the DNA was amplified for PCR follow the method in chapter II. The PCRs were analysed by 3% agarose gel electrophoresis and visualised by ethidium bromide on UV-transilluminator.

3.4 The Correlation of PCR and EOFT with Hb A_2 determination for diagnostic value

The α-thalassemia1 carriers which detected by PCR method were compared with the result from osmotic fragility test and HbA_2 determinant. At normal range of HbA_2 (≤4%), the α-thalassemia1 have osmotic fragility less than 60%. There was significant difference of osmotic fragility test between normal and α-thalassemia1 carrires that p value was 0.0001.

Table 5 Erythrocyte osmotic fragility values of normal women and of α-thalassemia 1 traits that have HbA_2 levels lower than 4%

Group	no. of samples	mean level of osmotic fragility (%)
normal	396	74.55 ± 20.91
α-thalassemia 1 -traits	37	32.81 ± 12.44

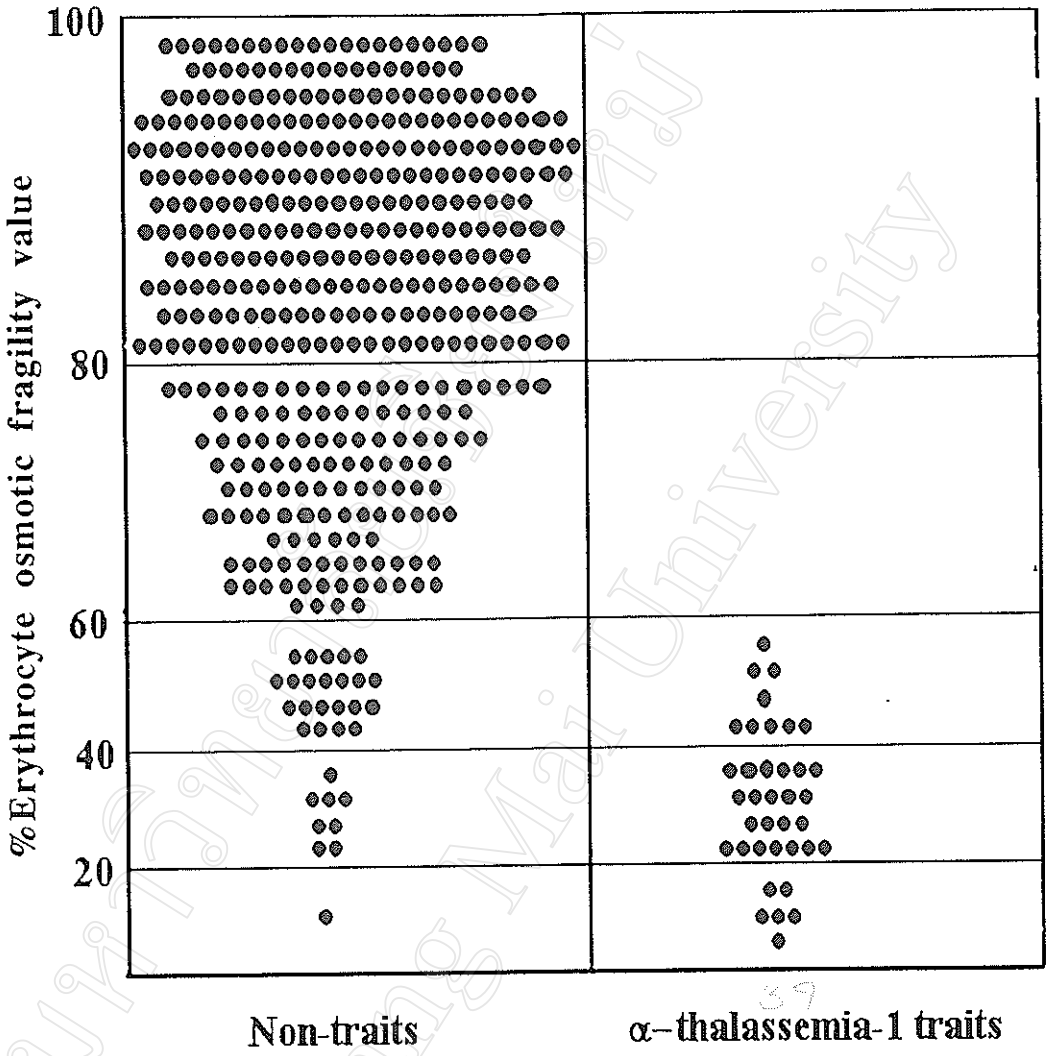

Figure 12 Erythrocyte osmotic fragility test values of non-traits and α-thalassemia 1 traits