CHAPTER IV

RESULTS AND DISCUSSION

There were 468 asymptomatic people involved as volunteers in this study for investigation for hemoglobinopathy trait. It consisted of 250 males and 218 females. Mean of age was 28.3 years and standard deviation was 9.5 years. The youngest sample was 14 years and the oldest was 72 years. Mostly were 14 to 38 years (see table 4.1).

Table 4.1. Frequency distribution of age

No.	Class limits	Freq.	Percent	Cumulative	
				Freq.	Percent
1	14.00 - < 20.00	85	18.16	85	18.16
2	20.00 - < 26.00	128	27.35	213	45.51
3	26.00 - < 32.00	105	22.44	318	67.95
4	32.00 - < 38.00	82	17.52	400	85.47
5	38.00 - < 44.00	34	7.26	434	92.74
6	44.00 - < 50.00	16	3.42	450	96.15
7	50.00 - < 56.00	12	2.56	462	98.72
8	56.00 - < 62.00	3	0.64	465	99.36
9	62.00 - < 68.00	2	0.43	467	99.79
10	68.00 - < 74.00	1	0.21	468	100.00
	Total	468	100.00		

Mean of hemoglobin level was 13.97 g/dL and standard deviation was 1.6 g/dL. The lowest level was 9.2 g/dL and the highest was 18.8 g/dL. Mostly hemoglobin level showed between 11.12 and 16.88 g/dL (see table 4.2.). Symptom of anemia will appear when hemoglobin level fall below 7 - 8 g/dL. (Hillman and Ault, 1995). Therefore, data of hemoglobin level confirmed that they were really asymptomatic people.

Mean of hemoglobin level among those who suffered from β thalassemia trait was 11.9 g/dL. and standard deviation was 1.3 g/dL. The lowest level was 9.7 g/dL and the highest was 13.1 g/dL.

Mean of hemoglobin level among hemoglobin E trait cases was 13.3 g/dL. and standard deviation was 1.4 g/dL. The lowest level was 10.8 g/dL. and the highest was 16.9 g/dL. Reliability on hemoglobin measurement was good because coefficient of variation was 0.7 %.

Table 4.2. Frequency distribution of hemoglobin level

No.	Class limits	Freq.	Percent	Cumulative	
				Freq.	Percent
1	9.00 - < 10.00	3	0.64	3	0.64
2	10.00 - < 11.00	12	2.56	15	3.21
3	11.00 - < 12.00	34	7.26	49	10.47
4	12.00 - < 13.00	82	17.52	131	27.99
5	13.00 - < 14.00	99	21.15	230	49.15
6	14.00 - < 15.00	107	22.86	337	72.01
7	15.00 - < 16.00	77	16.45	414	88.46
8	16.00 - < 17.00	41	8.76	455	97.22
9	17.00 - < 18.00	11	2.35	466	99.57
10	18.00 - < 19.00	2	0.43	468	100.00
	Total	468	100.00		

There were 51 cases of hemoglobinopathy found in this study which consisted of 12 cases of β thalassemia (2.6 %) and 31 cases of hemoglobin E trait (8.3 %). The prevalence of β thalassemia trait was not different too much to the previous findings. Boon (1983) estimated that the prevalence of β thalassemia trait was 3 % and around 3 to 7.8 % according to Sofro et al. (1986); Pramudji el al. (1991) and Wahidiyat (1991).

The prevalence of hemoglobin E trait was higher than 4 % that was estimated by Boon in 1983. Its prevalence was

also higher than β thalassemia trait. Therefore, the probability of having a baby who suffer from double heterozygous β thalassemia/Hb E is higher than that with homozygous β thalassemia. WHO working group (1982) found similar finding and they stated that β thalassemia/hemoglobin E occurred widely throughout southeast Asia. They also explained that β thalassemia/hemoglobin E was more important than homozygous β thalassemia because its incidence was higher and the patients could live longer.

Among those who suffered from hemoglobin E trait, 20 cases were male and 19 cases were female. Four of them (1 male and 3 females) were typical case of hemoglobin E trait because they showed MCH values are normal but the other were not. Mean of MCV and MCH values were respectively 75.7 fL. and 24.6 pg. The lowest value of MCV was 67.2 fL. and the highest was 83.6 fL. whereas the lowest value of MCH was 21 pg. and the highest was 30.7 pg.

There were 4 cases of microcytosis that still could not be classified into hemoglobinopathy. They might be α thalassemia trait or other type of thalassemia. For confirmation it needs the further investigation of microcytic history among their each families. Unfortunately, it had not been done due to geographical constraint and time limitation. They were microcytic and hypochromic people, hemoglobin A_2 and

ferritin level were normal (see table 4.3.). And if we look at the standard screening procedure (appendix 3) they are most likely to be α thalassemia or other type of thalassemia.

Table 4.3. Laboratory data of microcytosis cases

MCV (fL.)	MCH (pg.)	Hb A_2 (%)	Ferritin (ng/mL.)
76.6	23.6	3.3	81.3
76.1	25.9	2.0	105.8
71.7	24.0	1.8	78.2
78.5	26.0	1.8	57.3

Those people who suffered from iron deficiency and iron deficiency anemia were respectively 31 (6.6 %) and 20 (4.3 %). Eight of them were male and the other (44) were female. It was also known that the two of them (code number 16 and 100) were regular blood donor. This unexpected finding can be used as a basis for further study of prevalence of iron deficiency/ iron deficiency anemia among regular blood donor. Then the policy of blood donation might be reevaluated if its prevalence was high.

There were 3 cases of polycythemia; 2 cases of echinocytosis; 3 cases of ovalocytosis; 1 case of hereditary persistent fetal hemoglobin (HPFH), 1 case of hypoplastic anemia and 369 of normal people. The complete findings shown in table 4.4.

Table 4.4. Characteristic of the study population

Description	Number	Percent
Hemoglobinopathy	51	10.9
β thalassemia trait	12	2.6
Hemoglobin E trait	39	8.3
Iron deficiency	31	6.6
Iron deficiency anemia	20	4.3
Microcytosis	4	0.9
Polycythemia	3	0.6
Echinocytosis	3	0.6
Ovalocytosis	2	0.4
HPFH	3	0.6
Hypoplastic anemia	1	0.2
Normal	369	78.8

THE MODIFIED O.F.T.

In this study the modified O.F.T. was validated with the standard screening procedure to detect hemoglobinopathies. The study population was classified into two groups namely hemoglobinopathy and non-hemoglobinopathy group. Those who suffered from iron deficiency, iron deficiency anemia, microcytosis, polycythemia, echinocytosis, ovalocytosis, hypoplastic anemia and those normal people were classified as non-hemoglobinopathy group. It was found 51 cases of hemoglobinopathy and 417 people were classified into non-hemoglobinopathy group (see table 4.5.).

Table 4.5. A validation of the modified O.F.T. to the standard screening procedure

	Hemoglobinopathy		Non-hemoglobinopathy		
Test positive	44	a	b	103	147
Test negative	7	c	d	314	321
	51			417	468

People who had positive test result were 147 and those who had a negative test result were 321. Number of true positive result was 44, true negative result was 314, false positive result was 103 and false negative result was 7. All of cases of echinocytosis, ovalocytosis and HPFH showed false positive result. False positive test results were also found in 39.2 % of iron deficiency/iron deficiency anemia, 66.7 % of polycythemia, 75 % of microcytosis and 19 % of normal people. Whereas all of false negative test result were hemoglobin E trait.

Diagnostic performance of the modified O.F.T. in study population which has 10.9 % prevalence of hemoglobinopathy is as follows : sensitivity is 86.3 %, specificity is 75.3 %, positive predictive value (PPV) is 29.9 %, negative predictive

value (NPV) is 97.8 % and accuracy is 76.5 %. Diagnostic performance of this test at 95 % confidence interval can be seen in table 4.6.

Table 4.6. Diagnostic performance of the modified O.F.T. at 95 % confidence interval.

Test performance	Percent	95 % CI (%)
Sensitivity	86.3	82.9 - 89.7
Specificity	75.3	73.3 - 77.3
PPV	29.9	26.6 - 33.2
NPV	97.8	97.6 - 98.0
Accuracy	76.5	74.9 - 78.1

Suppose 4 cases of microcytosis were included into hemoglobinopathy group (they were very likely to be in this group), diagnostic performance of the modified O.F.T. a little bit change to be as follows : sensitivity is 85.5 %, specificity is 75.8 %, PPV and NPV are respectively 32 % and 97.5 %, accuracy is 76.9 % and prevalence is 11.8 %.

A positive test is 3.5 times more likely to be made in the present of hemoglobinopathy than in the absence of it (LR + = 3.5). If the test result was negative, the likelihood ratio for this negative test (LR -) was 0.2; the odds were about 1:6.1 that a negative test result would be made in the presence of hemoglobinopathy as compared to the absence of it.

The pre-test odds is 0.1 and the post-test odds is 0.4.

It was mentioned in primary research question that the modified O.F.T. would be accepted if it had a sensitivity at least 80 %. Lower limit of sensitivity of the modified O.F.T. at 95 % confidence interval is 82.9 %. Therefore, it is acceptable and can be used as an alternative for preliminary test for screening for hemoglobinopathies. It is supported by WHO (1983) that recommended to use a simple O.F.T. for initial mass screening for thalassemia.

Sixty six samples were tested blindly by two observers. The first observer found that 19 of them had positive test result and 47 of them had negative test result. Whereas the second observer found that 20 of them had positive test result and 46 of them had negative test result. The discrepancy result among these two observer was found in 5 samples (see table 4.7.). Inter observer agreement of this test is categorized almost perfect because the kappa value is 0.82 (Sackett et al., 1991).

Table 4.7. Inter observer agreement of the modified O.F.T.

		Test result of the 1st observer		
		Positive	Negative	
Test result of the 2nd observer	Positive	17	3	20
	Negative	2	44	46
		19	47	66

THE PREVIOUS O.F.T.

Mean of hemolysis level was 87.7 % and standard deviation is 9.2 %. The lowest level was 40.3 % and the highest was 99.5 %. Number of those who had less than 91 % hemolysis level was 232 people and the other (236 people) had equal or more than 91 % (see table 4.8.).

Table 4.8. Frequency distribution of hemolysis level

No.	Class limits	Freq.	Percent	Cumulative Freq.	Cumulative Percent
1	40.00 - < 50.00	3	0.64	3	0.64
2	50.00 - < 60.00	7	1.50	10	2.14
3	60.00 - < 70.00	10	2.14	20	4.27
4	70.00 - < 80.00	55	11.75	75	16.03
5	80.00 - < 90.00	147	31.41	222	47.44
6	90.00 - < 100.00	246	52.56	468	100.00
	Total	468	100.00		

In this study the previous O.F.T. was also validated with a standard screening procedure to detect hemoglobinopathies. The study population was also classified into two groups i.e. hemoglobinopathy and non-hemoglobinopathy group. Those who suffered from iron deficiency, iron deficiency anemia, microcytosis, polycythemia, echinocytosis, ovalocytosis, hypoplastic anemia and those normal people were classified as non-hemoglobinopathy group. The same as the previous comparison, number of those who classified into hemoglobinopathy group were 51 cases and 417 people were classified into non-hemoglobinopathy group (see table 4.9.).

Number of people who had positive test result were 232 and those who had negative test result were 236. Number of true positive result was 45, true negative result was 230, false positive result was 187 and false negative result was 6. All cases of echinocytosis, ovalocytosis and polycythemia showed false positive result. False positive result were also found in 41.3 % of iron deficiency/iron deficiency anemia, 33.3 % of HPFH, 75 % of microcytosis and 41.2 % of normal people. Whereas, the false negative results consisted of 4 cases of hemoglobin E trait and 2 cases of β thalassemia trait.

Table 4.9. A validation of the previous O.F.T. to the standard screening procedure

	Hemoglobinopathy	Non-hemoglobinopathy	
Test positive	45 a	b 187	232
Test negative	6 c	d 230	236
	51	417	468

Computation results of diagnostic performance of the modified O.F.T. in the study population which has 10.9 % prevalence of hemoglobinopathy are as follows : sensitivity is 88.2 %, specificity is 55.2 %, PPV and NPV are respectively 19.4 %, and 97.5 % as well as accuracy is 58.8 %. Diagnostic performance of this test at 95 % confidence interval can be seen in table 4.10.

Table 4.10. Diagnostic performance of the previous O.F.T. at 95 % confidence interval.

Test performance	Percent	95 % CI (%)
Sensitivity	88.2	85.4 - 91.0
Specificity	55.2	52.8 - 57.6
PPV	19.4	17.4 - 21.4
NPV	97.5	97.2 - 97.8
Accuracy	58.8	56.6 - 61.0

A positive test is 1.96 times more likely to be made in the present of hemoglobinopathy than in the absence of it (LR + = 1.96). If test result was negative, the likelihood ratio for this negative test result was 0.2; the odds were about 1:7.3 that a negative test result would be made in the presence of hemoglobinopathy as compared to the absence of it. The pre-test odds is 0.12 and the post-test odds is 0.2.

Suppose 4 cases of microcytosis were included into hemoglobinopathy group (they were very likely to be in this group), diagnostic performance of the modified O.F.T. a little bit change to be as follows : sensitivity is 87.3 %, specificity is 55.4 %, PPV and NPV are respectively 20.7 % and 97.0 %, accuracy is 59.2 % and prevalence is 11.8 %.

There were 20 tubes containing 200 ul heparinized blood collected from one person. Ten tubes were given to laboratory technician and the other 10 tubes to another technician. These samples were tested blindly by the two laboratory technician using the previous O.F.T. Reliability in this measurement between these two technicians was computed from their test results and expressed by intraclass coefficient correlation (ICC). Finally ICC was found 0.68, it means that the agreement among them is good.

Mean of hemolysis level among hemoglobinopathy cases was 76.1 % and standard deviation was 12,9 %. The lowest level was 43.4 and the highest was 94.4 %. Complete finding can be seen in table 4.11.

Table 4.11. Frequency distribution of hemolysis level among hemoglobinopathy cases

No.	Class limits	Freq.	Percent	Cumulative	
				Freq.	Percent
1	40.00 - < 50.00	2	3.92	2	3.92
2	50.00 - < 60.00	5	9.80	7	13.73
3	60.00 - < 70.00	4	7.84	11	21.57
4	70.00 - < 80.00	20	39.22	31	60.78
5	80.00 - < 90.00	11	21.57	42	82.35
6	90.00 - < 100.00	9	17.65	51	100.00
	Total	51	100.00		

COMPARISON BETWEEN THE MODIFIED AND THE PREVIOUS O.F.T.

Twenty one of 51 cases of iron deficiency/iron deficiency anemia (41.2 %) give false positive result to the previous O.F.T. whereas in the modified O.F.T. only 20 people (39.2%). All of 3 echinocytosis cases and 2 cases of ovalocytosis give false positive result to both the modified and the previous O.F.T. One hundred and fifty four of 369 normal people (41.7 %) give false positive result to the

previous O.F.T. whereas in the modified O.F.T. only 70 people or 19 % (see table 4.12.). In general, the previous O.F.T. shows that 84.2 % of total false positive result are normal people whereas the modified O.F.T. shows only 68 percent. If the previous O.F.T. was chosen as a preliminary test for screening for hemoglobinopathies cost would be more than the modified O.F.T. due to unnecessary tests.

Table 4.12. A comparison between the modified and the previous O.F.T. based on false positive result.

	Modified O.F.T.	Previous O.F.T.
Normal	70/369(19.0 %)	154/369(41.7 %)
Iron def./iron def. anemia	20/51 (39.2 %)	21/51 (41.2 %)
Echinocytosis	3/3 (100 %)	3/3 (100 %)
Ovalocytosis	2/2 (100 %)	2/2 (100 %)
Microcytosis	3/4 (75 %)	3/4 (75 %)
Polycythemia	2/3 (66.7 %)	3/3 (100 %)
HPFH	3/3 (100 %)	1/3 (33.3 %)
Total	103	187

All of false negative result detected by the modified O.F.T. are hemoglobin E trait. Whereas, false negative result detected by the previous O.F.T. consisted of 4 cases of hemoglobin E trait and 2 cases of β thalassemia trait. Even though number of false negative result was higher in the

modified O.F.T. but there were no case of β thalassemia trait lost from screening (see table 4.13.).

Table 4.13. A comparison between the modified O.F.T. and the previous O.F.T. based on the false negative result.

	Modified O.F.T.	Previous O.F.T.
Hemoglobin E trait	7 cases	4 cases
β thalassemia trait	0	2 cases

Number of positive and negative test result detected by the modified O.F.T. were respectively 147 and 321. Whereas number of positive and negative test result detected by the previous O.F.T. were respectively 232 and 236. Number of positive test result detected by both the modified and the previous O.F.T. was 122. Number of negative test result detected by both the modified and the previous O.F.T. was 211. And there were 135 discrepancy test result between these two tests (see table 4.14). Statistical analysis by Chi-square test showed that these two test were significantly different in term of detection rate ($p < 0.05$).

Table 4.14. A comparison between the modified and the previous O.F.T. based on detection rate

		The modified O.F.T.		
		Positive	Negative	
The previous O.F.T.	Positive	122	110	232
	Negative	25	211	236
		147	321	468

Sensitivity of the previous O.F.T. is 88.2 %. It is better than the modified O.F.T. Sensitivity of these two tests are still acceptable because their sensitivity are more than 80 % (see research question and sample size calculation). However, the modified O.F.T. looks better than the previous O.F.T. because the other characteristic of the modified O.F.T. is better (see table 4.15.).

Table 4.15. A Comparison between the modified O.F.T. and the previous O.F.T.

Test performance	Modified O.F.T.	Previous O.F.T.
Sensitivity	86.3	88.2
Specificity	75.3	55.2
PPV	29.9	19.4
NPV	97.8	97.5
Accuracy	76.5	58.8
L R (+)	3.5	2.0
L R (-)	0.2	0.2
Pre-test odds	0.1	0.1
Post-test odds	0.4	0.2

TOTAL BILIRUBIN LEVEL

Mean of total bilirubin level was 0.77 mg/dL and standard deviation was 0.4 mg/dL. The lowest level was 0.1 mg/dL and the highest level was 3.3 mg/dL. Normally total bilirubin level in adult is 0.3 to 1.3 mg/dL. (IL cat. no. 35248). There were 38 people showed bilirubin level more than 1.3 mg/dL., whereas the other (430 people) showed bilirubin level equal or less than 1.3 mg/dL. Frequency dictribution of total bilirubin level can be seen in table 4.16.

Table 4.16. Frequency distribution of total bilirubin level

No.	Class limits	Freq.	Percent	Cumulative	
				Freq.	Percent
1	0.00 - < 0.50	85	18.16	85	18.16
2	0.50 - < 1.00	280	59.83	365	77.99
3	1.00 - < 1.50	71	15.17	436	93.16
4	1.50 - < 2.00	21	4.49	457	97.65
5	2.00 - < 2.50	7	1.50	464	99.15
6	2.50 - < 3.00	3	0.64	467	99.79
7	3.00 - < 3.50	1	0.21	468	100.00
	Total	468	100.00		

In the modified O.F.T., among those who showed bilirubin level more than 1.3 mg/dL. 10 people showed positive test result and 28 people showed negative test result. Among 430 of those who had total bilirubin level equal or less than 1.3 mg/dL., 137 people showed positive test result and 293 people showed negative test result (see table 4.17.). Statistical analysis result by using Chi-square test expresses that total bilirubin level more than 1.3 mg/dL. does not show any different compared with its normal level to influence the test result ($p > 0.05$).

Table 4.17. A comparison between total bilirubin level and test result of the modified O.F.T.

		Positive test	Negative test	
Total bilirubin level	> 1.3 mg/dL	10	28	38
	≤ 1.3 mg/dL	137	293	430
		147	321	468

In the previous O.F.T., among those who showed bilirubin level more than 1.3 mg/dL. 19 people showed positive test result and 19 people showed negative test result.. Among 430 of those who showed total bilirubin level equal or less than 1.3 mg/dL., 213 people showed positive result and 217 people showed negative test result (see table 4.18.). Statistical analysis by using Chi-square test expresses that total bilirubin level more than 1.3 mg/dL. does not also show any different compared with its normal level to influence the test result ($p > 0.05$).

Table 4.18. A comparison between total bilirubin level and test result of the previous O.F.T.

		Positive test	Negative test	
Total bilirubin level	> 1.3 mg/dL	19	19	38
	≤ 1.3 mg/dL	213	217	430
		232	236	468

MCV AND/OR MCH VALUES

Mean of MCV value was 84.03 fL. and standard deviation was 5.68 fL. The lowest value was 57.9 fL. and the highest value was 95.4 fL. Number of those who had MCV value less than 80 fL. were 86 people. Thirty three males had MCV value less than 79 fL. and 34 females had MCV value less than 77 fL. Complete finding can be seen in table 4.19.

Table 4.19. Frequency distribution of MCV value

No.	Class limits	Freq.	Percent	Cumulative Freq.	Cumulative Percent
1	50.00 - < 60.00	3	0.64	3	0.64
2	60.00 - < 70.00	11	2.35	14	2.99
3	70.00 - < 80.00	72	15.38	86	18.38
4	80.00 - < 90.00	346	73.93	432	92.31
5	90.00 - < 100.00	36	7.69	468	100.00
	Total	468	100.00		

Mean of MCH value was 27.89 pg. and standard deviation was 2.48 pg. The lowest value was 17.4 pg. and the highest value was 33.8 pg. Fifty two males had MCH value less than 27 pg. and 51 females had MCH value less than 26 pg. Complete finding can be seen in table 4.20.

Table 4.20. Frequency distribution of MCH value

No.	Class limits	Freq.	Percent	Cumulative	
				Freq.	Percent
1	17.00 - < 19.00	2	0.43	2	0.43
2	19.00 - < 21.00	7	1.50	9	1.92
3	21.00 - < 23.00	11	2.35	20	4.27
4	23.00 - < 25.00	39	8.33	59	12.61
5	25.00 - < 27.00	72	15.38	131	27.99
6	27.00 - < 29.00	164	35.04	295	63.03
7	29.00 - < 31.00	142	30.34	437	93.38
8	31.00 - < 33.00	28	5.98	465	99.36
9	33.00 - < 35.00	3	0.64	468	100.00
	Total	468	100.00		

There were 32 males who had MCV value less than 79 fL. and MCH value less than 27 pg.(microcytic and hypochromic). Twenty six of them (81.3 %) suffered from hemoglobinopathy. Whereas in the females sub-group 34 of them had MCV value less than 77 fL. and MCH value less than 26 pg. And 25 of them (73.5 %) suffered from hemoglobinopathy. Finally, can be concluded that all of hemoglobinopathy cases are microcytic and hypochromic. Number of males and females in microcytic and hypochromic sub-group are comparable (see table 4.21.).

Table 4.21. Prevalence of hemoglobinopathy among microcytic and hypochromic sub-group.

		Hemoglobinopathy	
	Microcytic & hypochromic	Number	Percent
Male	32	26	81.3
Female	34	25	73.5
Total	66	51	73.3

Reliability of both MCV and MCH values were excellent. It could be seen in their measurement expressed by coefficient of variation (CV). The CV of MCV and MCH measurements were respectively 0.34 % and 1.19 %.

HEMOGLOBIN A_2 DETERMINATION

Hemoglobin A_2 value among non-hemoglobinopathy group had mean 3.06 % and standard deviation was 0.35 %. The lowest value was 1.1 % and the highest value was 3.4 %. This finding was similar to the previous study that found that mean was 2.42 % and the range was 0.9 % - 3.32 % (Cao et.al., 1984).

Mean of Hb A_2 value among β thalassemia trait cases was 4.98 % and standard deviation was 0.8 %. The lowest value was 3.6 % and the highest value was 6.5 %. Whereas Cao et. al. (1984) found that the mean was 5.09 % and range was 3.5 - 7.3 %.

Mean of Hb A_2 value among those who suffered from hemoglobin E trait was 24.2 % and standard deviation was 3.2 %. The lowest value was 17 % and the highest value was 28.5 % (see table 4.22.).

Table 4.22. Result of Hemoglobin A_2 determination

	Mean (%)	SD (%)	Range (%)
Non hemoglobinopathy	3.06	0.35	1.10 - 3.40
Hemoglobinopathy			
β thalassemia trait	4.98	0.80	3.60 - 6.50
Hemoglobin E trait	24.20	3.20	17.00 - 28.00

Reliability of Hb A_2 measurement was determined from an inter observer agreement. Between two observers that involved in this step, they showed a good agreement because intra class correlation coefficient was 0.64.

HEMOGLOBIN F DETERMINATION

Mean of hemoglobin F value among β thalassemia trait cases was 2.5 % and standard deviation was 0.6 %. The lowest value was 1.9 % and the highest value was 3.8. And for those who suffered from HPFH, the mean of hemoglobin F was 5.5 %, standard deviation was 1.3 % (range 4.4 - 6.9).

HEMOGLOBIN ELECTROPHORESIS

Result of hemoglobin electrophoresis on cellulose acetate membrane of those who did not have any abnormalities showed bands of hemoglobin A and A_2 fraction. Those who suffered from β thalassemia trait showed hemoglobin fraction A, A_2 and tiny band of hemoglobin F fraction. Hemoglobin E and A_2 took place tightly and made the band become bigger. This pattern could be seen in all of those who suffered from hemoglobin E trait.

FERRITIN LEVEL DETERMINATION

Ferritin level had been measured among 86 people who had MCV value less than 80 fL. Thirty five of them were male and 51 of them were female. Among the male group, 8 people suffered from iron deficiency, 27 people were normal and none of them suffered from iron deficiency anemia. In the female sub-group 21 people of them suffered from iron deficiency, 19 people suffered from iron deficiency anemia and 11 of them were normal. The mean of ferritin level in this group was 44.9 ng/mL and standard deviation was 40.8 ng/mL. The lowest value was 3.4 ng/mL., the highest level was 232.9 ng/mL. and there was no an extreme value. In general, 22.1 % of them suffered from iron deficiency anemia and 33.7 % suffered from iron deficiency whereas the rest (44.2 %) showed normal value

(see table 4.23.).

However, there were 3 people who had MCV value equal or more than 80 fL. (range 80 - 80.5 fL.) also suffered from iron deficiency/iron deficiency anemia. Two of them (code number 93 and 402) suffered from iron deficiency and another (code number 177) suffered from iron deficiency anemia. So that in the study population, total number of people who suffered from iron deficiency and iron deficiency anemia were respectively 31 and 20 people.

Table 4.23. Ferritin level among those who had MCV value less than 80 fL.

	Sex		Total	Percent
	Male	Female		
Iron deficiency	8	21	29	33.7
Iron deficiency anemia	0	19	19	22.1
Normal	27	11	38	44.2
Total	35	51	86	100.0

RBC MORPHOLOGY

RBC morphology was read from peripheral blood smear. Anisocytosis and poikilocytosis can be seen in β thalassemia trait group. Pencil cell usually present among those who also suffer from iron deficiency/iron deficiency anemia.

Most of those who suffered from hemoglobin E trait showed microcytic. Some of them showed filamented RBC. And some of them who also suffered from iron deficiency/iron deficiency anemia showed pencil cell as well.

Microcytic, anisocytosis and poikilocytosis were frequently seen in those who suffered from iron deficiency/iron deficiency anemia. Pencil cell was a good marker for this disease. Sometimes it was difficult to differentiate between β thalassemia trait and this disease by RBC morphology examination only.

All of ovalocytosis and echinocytosis cases showed false positive test result. In the case of echinocytosis it can be realized because surface and volume ratio of RBC is higher than that in normal people.

Ovalocytosis is a disease that also can be inherited. The defect is in skeletal protein of RBC's membrane. In southeast Asia it is known as southeast Asia or Melanesian ovalocytosis. It was found in up to 30 % of aboriginal population of coastal areas of Papua New Guinea or Malaysia (Pelek, J., 1991). The two of ovalocytosis cases were from Irian Jaya province (east part of Indonesia). This place is nearby Papua New Guinea. Therefore, they most likely are Melanesian ovalocytosis. And it can be assumed that

resistance to hypotonic saline solution of their RBC due to defect on their RBC's membrane.