CHAPTER 3

Results

3.1 The production of antinuclear antibodies (ANA) in HIV-infected and healthy individuals

Samples from 76 HIV-infected and 100 healthy individuals were subjected to ANA determination using immunofluorescence technique.

All HIV-infected patients had started receiving combination antiretroviral therapy (cART) for at least 1 year at Maeon Hospital and had maintained viral load at less than 50 copies/mL (undetectable) at the time of blood collection. These participants were at the age between 18 to 60 that have neither the history of autoimmune diseases nor opportunistic infections (OIs) and were not pregnant. Of 76 HIV-infected patients, 29 (38.2%) were male and 47 (61.8%) were female. The averages of age, number of CD4 cells and the duration of HIV infection of the patients were 45.2 years, 529.4 cells/μL and 12.6 years, respectively (table 3.1, 3.2 and figure 3.1). Out of 76 patients, 19 were positive for ANA, representing a prevalence of 25% with 9 (31.0%) male and 10 (21.3%) female. The prevalence of ANA positive in patients seemed to be higher in male but the difference did not reach statistical significance (p-value = 0.340) (table 3.3). For the age, there was no statistically significant difference between ANA positive and negative group (p-value = 0.431). However, the highest ANA prevalence was presented in the 51- to 60-year group (figure 3.2). Moreover, there was no statistically significant difference of CD4 cell count, %CD4 and infection duration between ANA positive and negative group (p-value = 0.976, 0.746 and 0.125, respectively). For the infection duration, the highest ANA prevalence was presented in the 20- to 25-year group (figure 3.3). Among the patients with ANA positive result, there were 13 (68.4%) patients with a titer of 80 (2 homogeneous, 7 nucleoplasm granular, 3 mitotic and 1 for nucleoli positive patterns), 1 (5.3%) with a titer of 160

(nucleoplasm granular), 2 (10.5%) with a titer of 320 (1 homogeneous and 1 nucleoli positive) and 3 (15.8%) with a titer more than 1280 (1 homogeneous and 1 nucleoplasm granular) (table 3.4, table 3.5 and figure 3.4).

In addition, HIV-negative blood samples from healthy individuals were collected from 100 donors of Maharaj Nakorn Chiang Mai Hospital, 67 (67%) were male and 32 (32%) were female. The average age was 32.7 years (table 3.1, 3.2 and figure 3.1). Out of 100 donors, 22 were positive for ANA, representing a prevalence of 22% with 12 (17.6%) male and 10 (31.2%) female. The prevalence of ANA positive in donors was higher in female, though the difference did not reach statistical significance (p-value = 0.126) (table 3.3). For the age, there was no statistically significant difference between ANA positive and negative group (p-value = 0.72) but the highest ANA prevalence was presented in the 51- to 60-year group (figure 3.2). Among the donors with ANA positive result, there were 15 donors with a titer of 80 (5 homogeneous, 8 granular, 1 dotted, 1 mixed (homogeneous and granular), 3 with a titer of 160 (2 granular and 1 nucleoli), 1 with a titer of 640 (granular) and 3 with a titer of > 1280 (3 nucleoli) (table 3.4, table 3.5 and figure 3.4).

When compare between patient and donor group, for the age, donors (32.7 years) showed the significantly younger than patients (45.2 years) (p-value < 0.01) (table 3.1, 3.2 and Figure 3.1). However, for the ANA prevalence (HIV patients = 25%, Donors = 22%), there was no statistically significant difference among them (P-value = 0.641) (table 3.6).

Table 3.1 Subjects characteristics

Characteristics	Patients (n=76)	Healthy donors (n=100)
Age (yrs)*	45.2 (24-60)	32.7 (19-58)
Sex Male	29 (38.2%)	68 (68%)
Female	47 (61.8%)	32 (32%)
Infection duration (yrs)*	12.6 (1-25)	-
CD 4 (cells/μL) *	529.4 (149-1201)	-
%CD4*	24.8 (9-43)	-
Viral load (copies/mL)	< 50 (undetectable)	-

*The characteristics were presented as mean with range in the bracket

Table 3.2 Distribution by gender and age group of subjects

Age group	Patients (n=76)			Healthy donors (n=100)		
	male	female	total	male	female	total
< 20	0	0	0	9	2	11
21-30	1	1	2	22	12	34
31-40	7	8	15	22	9	31
41-50	13	29	42	12	7	19
51-60	8	9	17	3	2	5
Total	29	47	76	68	32	100

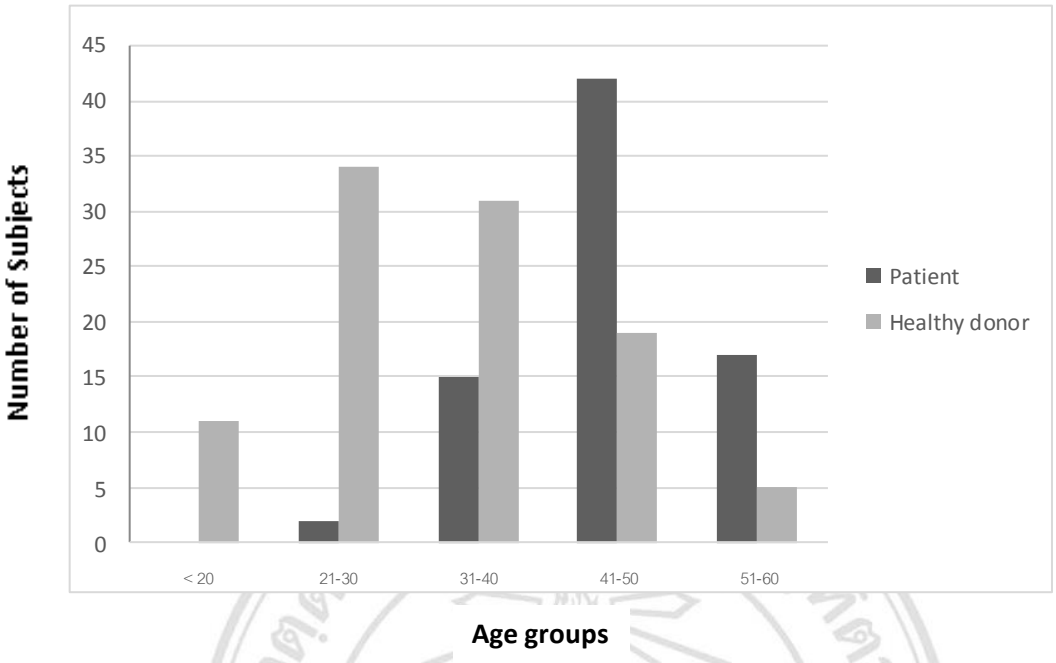


Figure 3.1 Total number of Subjects (HIV infected patients and healthy blood donors), distributed according to age group.

Table 3.3 Prevalence of ANA positive of the subjects, according to gender

ANA (Count and **%** within Sex)	**Patients (n=76)**			**Healthy donors (n=100)**		
	male	female	total	male	female	total
Negative	20 (69.0%)	37 (78.7%)	57 (75.0%)	56 (82.4%)	22 (69.8%)	78 (78.0%)
Positive	9 (31.0%)	10 (21.3%)	19 (25.0%)	12 (17.6%)	10 (31.2%)	22 (22.0%)
Total	29 (100.0%)	47 (100.0%)	76 (100.0%)	68 (100.0%)	32 (100.0%)	100 (100.0%)

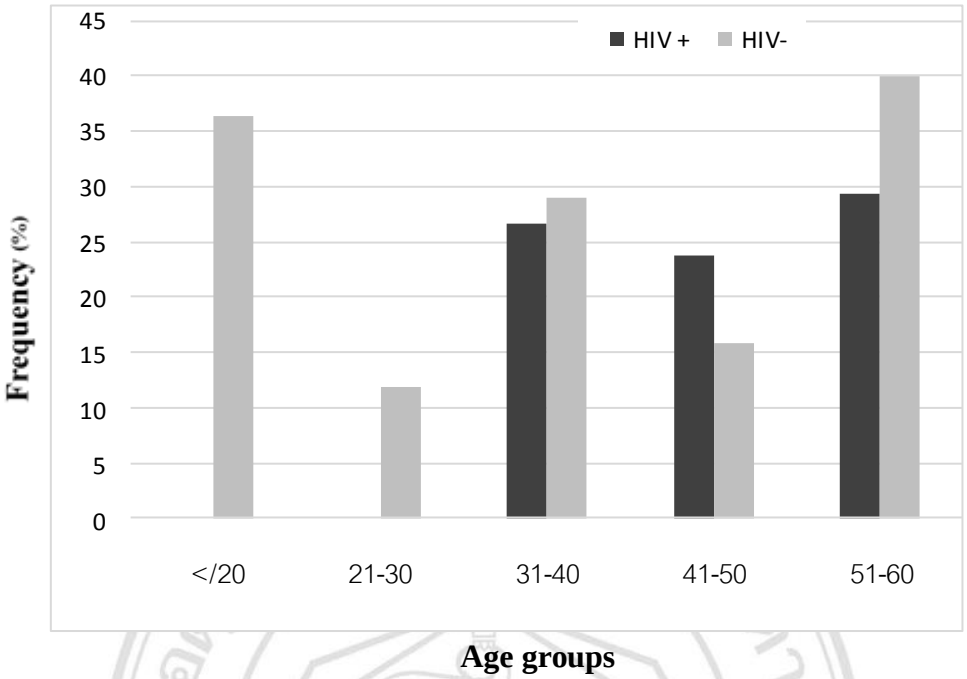


Figure 3.2 Prevalence of ANA positive in subjects, according to age groups.

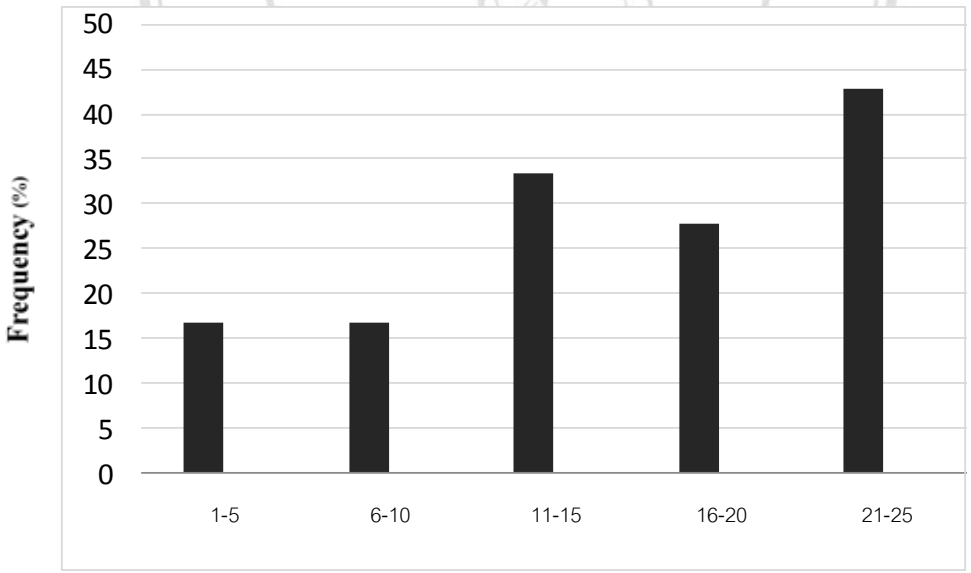


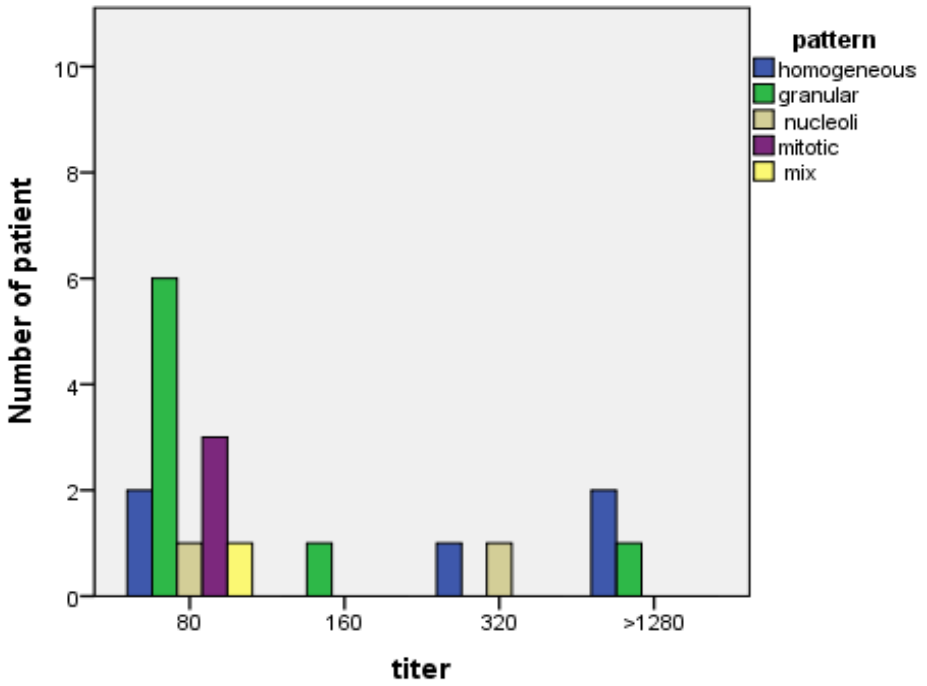
Figure 3.3 Prevalence of ANA positive in HIV patients, according to infection duration.

Table 3.4 Prevalence of ANA positive of the Subjects, according to different titers

ANA (Titer)	Patients (n=19)			Healthy donors (n=22)		
	Male	Female	Total	Male	Female	Total
80	6	7	13 (17.1%)	8	7	15 (15%)
160	1	0	1 (1.3%)	2	1	3 (3%)
320	1	1	2 (2.6%)	0	0	0
640	0	0	0	0	1	1 (1%)
1280	1	2	3 (3.9%)	2	1	3 (3%)

Table 3.5 Prevalence of ANA positive of subjects, according to titers and pattern

Pattern	Patients (n=19)						Healthy donors (n=22)					
	Titers						Titers					
	80	160	320	640	>1280	Total	80	160	320	640	>1280	Total
Homogeneous	2	0	1	0	2	5	5	0	0	1	0	6
Granular	6	1	0	0	1	8	8	2	0	0	0	10
Nucleoli	1	0	1	0	0	2	0	1	0	0	3	4
Mitotic	3	0	0	0	0	3	1	0	0	0	0	1
Mix pattern	1	0	0	0	0	1	1	0	0	0	0	1
Total	13	1	2	0	3	19	14	3	0	1	3	22



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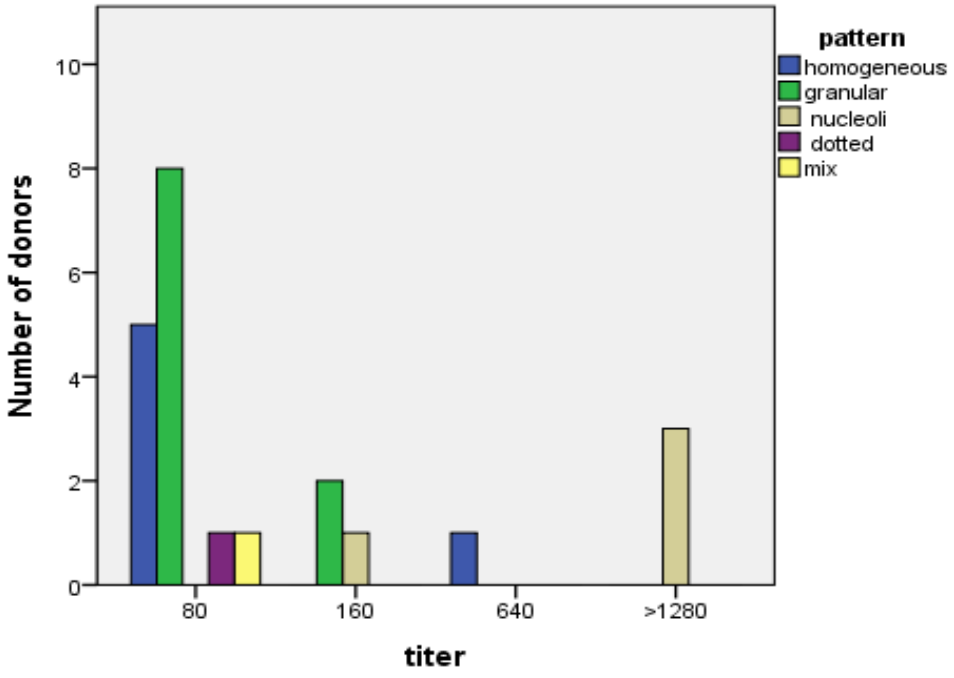

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Figure 3.4 Prevalence of ANA positive, according to pattern and titers, for patients (1) and donors (2).

Table 3.6 Subjects characteristics based on ANA result (positive/negative)

ANA	Patients (n=76)						Healthy donors (n=100)		
	Male	Female	Age (years)	CD4 (cell/µL)	%CD4	Infection Duration(yrs)	male	female	Age (years)
Negative	20	37	44.8	524.9	24.4	12.1	56	22	32.4
Positive	9	10	46..4	542.7	25.8	14.3	12	10	33.9

*The characteristics were presented as mean and statistically significant difference was only found between patient and donor aged (p-value < 0.01)

3.2 Percentage of T helper 17 cells and T regulatory cells

After ANA analysis, the samples were separated into 4 groups randomly based on HIV infection and ANA result for Th17 and Treg determination by flow cytometry as the following; 1) HIV negative with ANA negative (n=15), 2) HIV negative with ANA positive (n=11), 3) HIV positive with ANA negative (n=15), and 4) HIV positive with ANA positive (n=11). The averages of age were 36.0, 36.1, 45.0 and 47.9 for group 1, 2, 3 and 4, respectively. The HIV-infected participants were significantly older than the uninfected donor group (p-value <0.03). In addition, there was no statistically significant difference of CD4 cell count or CD4 percentage in HIV patient group (group 3 and 4) (p-value = 0.622 and 0.876, respectively) but infection duration in group 4 was higher than group 3 at p-value = 0.033. The characteristics of the subjects in each group are shown in table 3.7. For ANA positive result (group 2 and 4), most subjects were positive for ANA at low titer (1:80) with granular and homogeneous pattern (table 3.8 and 3.9). The average percentage of T helper 17 cells was 1.73, 1.65, 1.59 and 0.95 for group 1, 2, 3 and 4, respectively (table 3.10 and figure 3.5). There was no statistically significant difference among them (p-value = 0.057). Relative mean fluorescence intensity (MFI) of Th17 were also investigated (the relative MFI was calculated by divided the MFI of $CD3^+$ $CD8^-$ $IL\text{-}17^+$ cells with the MFI of $CD3^+$ $CD8^-$ $IL\text{-}17^-$ cells), the median was 4.34, 5.17, 9.32 and 6.47 (figure 3.6). The relative MFI of HIV infected groups trend to be higher than donor groups but only group 3 showed the significant difference to group 1 and group 2 (p-value < 0.01). The average percentages of Treg cells in those 4 groups were 3.89, 3.25, 3.35

and 3.94, respectively (table 3.10 and figure 3.5). The statistically significant difference was not found among them (p-value = 0.749). In addition, the average of Th17 and Treg ratio was 0.70, 0.83, 0.49 and 0.30 for group 1, 2, 3 and 4, respectively (table 3.10 and figure 3.5). The statistically significant difference also not found (p-value = 0.06).

Table 3.7 Subjects characteristic in each group

Characteristics	**Group 1 (n=15)**	**Group 2 (n=11)**	**Group 3 (n=15)**	**Group 4 (n=11)**
Age (yrs)*	36.0 (24-51)	36.1 (20-58)	45.0 (36-57)	47.9 (40-60)
Sex Male	11 (73.3%)	5 (45.5%)	5 (33.3%)	6 (54.5)
Female	4 (26.7%)	6 (54.5%)	10 (66.7%)	5 (45.5)
Infection duration (yrs)*	-	-	10.5 (3-19)	15.5 (5-23)
CD 4 (cells/μL) *	-	-	544.0 (239-1092)	577.6 (259-1061)
% CD4*	-	-	24.3 (11-36)	25.3 (13-43)
Viral load (copies/mL)	-	-	< 50 (undetectable)	< 50 (undetectable)

*The characteristics were presented as mean with range in the bracket; Group1 = HIV negative with ANA negative, Group2 = HIV negative with ANA positive, Group3 = HIV positive with ANA negative and group 4 HIV positive with ANA positive

Table 3.8 Prevalence of ANA positive of subjects (group 2 and 4), according to different titers

ANA (Titer)	**Group 2 (n=11)**			**Group 4 (n=11)**		
	Male	Female	Total	Male	Female	Total
80	2	4	6 (54.5)	4	4	8 (72.7)
160	2	1	3 (27.3)	0	0	0
320	0	0	0	1	0	1 (9.1)
>1280	1	1	2 (18.2)	1	1	2 (18.2)

Table 3.9 Prevalence of ANA positive of subjects (group 2 and 4), according to titers and pattern

Pattern	Group 2 (n=11)				Group 4 (n=11)			
	Titers				Titers			
	80	160	>1280	Total	80	320	>1280	Total
Homogeneous	1	0	0	1 (9.1)	2	0	1	3 (27.3)
Granular	4	2	0	6 (54.5)	4	0	1	5 (45.5)
Nucleoli	0	1	2	3 (27.3)	1	1	0	3 (27.3)
Mitotic	0	0	0	0	1	0	0	1 (9.1)
Mix pattern	1	0	0	1 (9.1)	0	0	0	0
Total	6	3	2	11	8	1	2	11

Table 3.10 Th17, Treg percentage and Th17/Treg ratio

No.	Group 1 (n=15)			Group 2 (n=11)			Group 3 (n=15)			Group 4 (n=11)		
	Th17	Treg	Ratio	Th17	Treg	Ratio	Th17	Treg	Ratio	Th17	Treg	Ratio
1	1.9	3.4	0.56	1.7	1.2	1.42	0.5	1.0	0.50	0.9	0.9	1.00
2	2.3	1.8	1.28	1.9	2.3	0.83	0.7	.9	0.78	0.3	4.5	0.06
3	1.1	3.6	0.31	1.6	2.0	0.80	1.9	4.5	0.42	0.6	3.5	0.17
4	2	1.6	1.25	2.8	1.2	2.33	1.1	3.3	0.33	0.3	5	0.06
5	2.3	4.5	0.51	0.9	1.3	0.69	0.4	2.7	0.15	0.9	3.4	0.26
6	1.1	1.4	0.79	2.1	1.4	1.50	1.7	2.2	0.77	1.5	4.3	0.35
7	1.4	5.4	0.26	1.0	5.8	0.17	2.8	5.0	0.56	0.4	6.1	0.06
8	2.7	5.4	0.5	1.4	6.5	0.22	2.9	3.5	0.83	1.8	4	0.45
9	3.5	2.1	1.67	1.7	6.9	0.25	1.8	3.4	0.53	0.3	3.4	0.09
10	1	6.5	0.15	1.4	3.5	0.40	1.5	4.7	0.32	2.1	4.9	0.43
11	2.5	1.2	2.08	1.7	3.6	0.47	2.5	4.1	0.61	1.4	3.4	0.41
12	0.6	1	0.6				2.5	3.6	0.69			
13	0.9	8.3	0.11				0.5	2.4	0.21			
14	1.8	5.2	0.35				1.1	3.7	0.30			
15	0.9	6.9	0.13				1.9	5.2	0.37			
Mean	1.73	3.89	.70	1.65	3.25	0.83	1.59	3.35	0.49	0.95	3.94	0.30

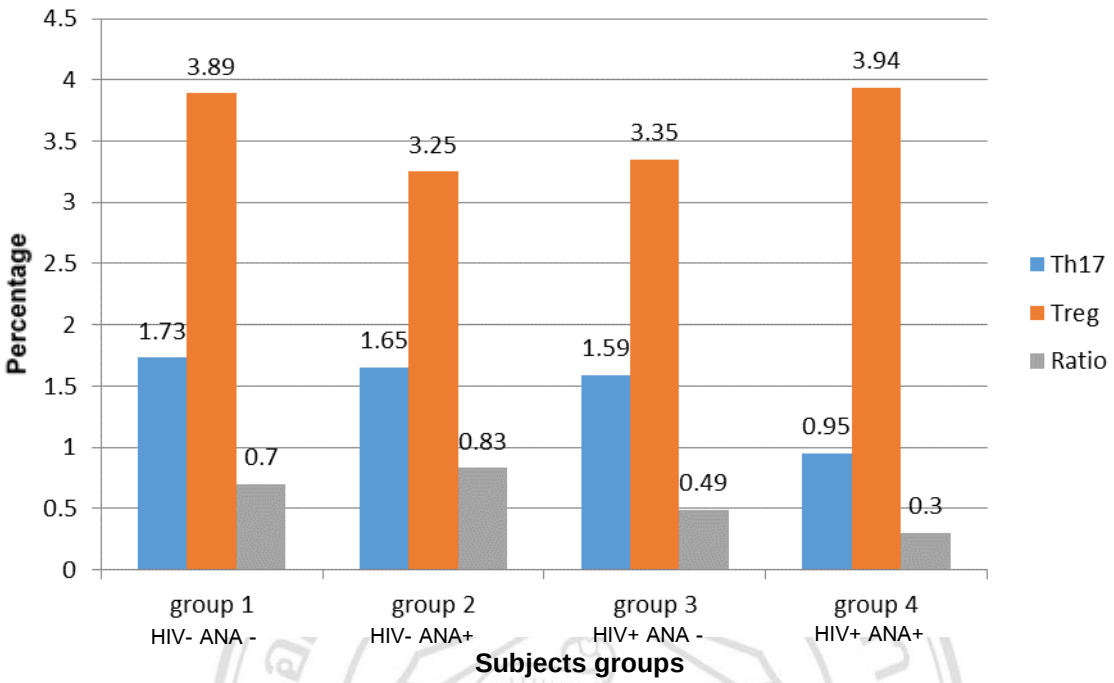

Figure 3.5 The average mean of Th17, Treg percentage and Th17/Treg ratio in 4 groups

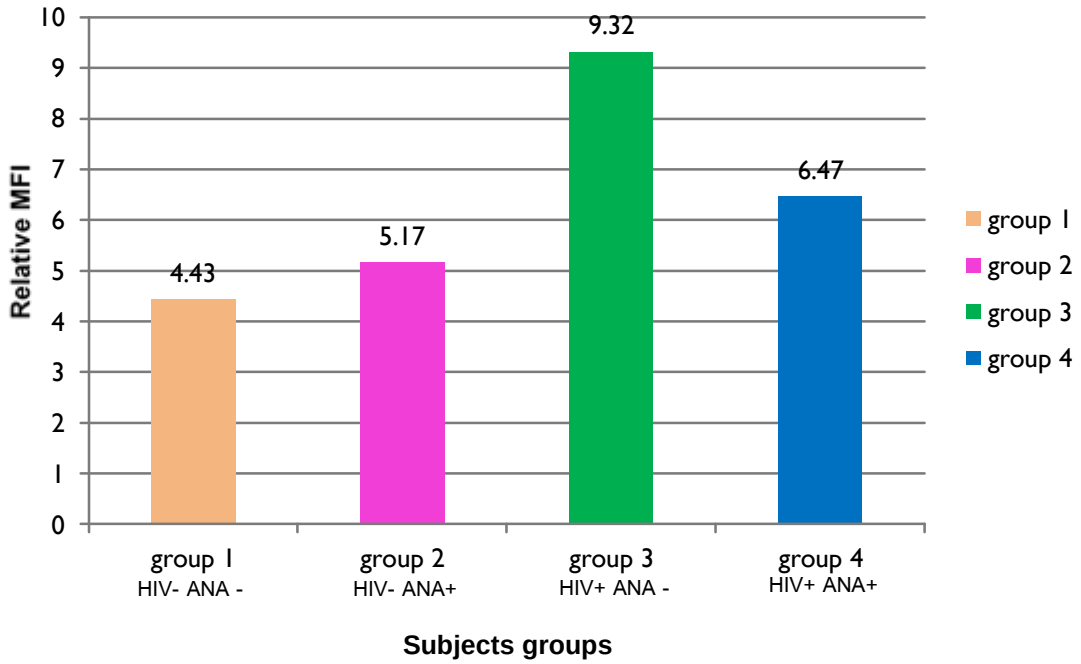

Figure 3.6 The median of relative MFI in 4 groups (the relative MFI was calculated by divided the MFI of $CD3^+$ $CD8^-$ $IL\text{-}17^+$ cells with the MFI of $CD3^+$ $CD8^-$ $IL\text{-}17^-$ cells)