INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 257
2020 July; 26(3): 257-265
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

The Association of Syphilis Infection and Other Risk Factors with Immunity of Patients with HIV on Anti-Retroviral Therapy

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ABSTRACT

Syphilis infection, age, gender, sexual behaviour, length of HIV infection and length of ARV treatment are suspected to be associated with amount of cluster of differentiation 4 ($CD4^+$)T-cells ($CD4^+$ T-cell) and $CD4^+$ T-cells: $CD8^+$ T-cells ratio (CD4:CD8) of HIV patients on ARV. However, the evidence remains lacking. The aim of this cross-sectional study on April 24th to June 20th, 2019 was to determine the association of syphilis infection and other risk factors (age, gender, sexual behavior, length of HIV infection and ARV treatment) with the immunity of patients with HIV on ARV based on T $CD4^+$ and CD4:CD8, and got factors those have an influence to T $CD4^+$ and CD4:CD8. Seventy-four subjects with age ≥18 years old with HIV on ARV from Voluntary Counselling Test (VCT) Outpatients of Dr. Moewardi General Hospital in Surakarta (DMGHS) had been examined for syphilis, T $CD4^+$ count and CD4:CD8 in Clinical Pathology Laboratory of DMGHS. Other data had been completed from the anamnesis and VCT data system. All data had been processed with SPSS version 21. Multivariate logistic regression following bivariate analysis of the Chi-Square test was used for categorical variables. Bivariate analysis showed a significant association between age, length of HIV infection and length of ARV treatment to T $CD4^+$ count and significant association between sexual behavior, length of HIV infection, and length of ARV treatment to CD4:CD8 ($p<0.05$). Multivariate analysis showed that the prevalence of Cd4 ≤ 500 was higher in male Prevalence Ratio (PR)=3.256; p=0.038) than that of female and subjects aged > 42 y.o. compared to those aged >18-42 y.o. (PR=3.451; p=0.047). The PR of CD4:CD8 < 0.3 in anal sex (PR=3.575; p=0.049) was higher than that of vaginal sex. The PR of CD4≤ 500 (PR=0.271; p=0.020) and CD4:CD8 < 0.3 (PR=0.125; p=0.001) in subjects with length of HIV > 5 years were lower than those in HIV 0-5 years. Age, gender, and length of HIV potentially affect the probability of T $Cd4^+$ ≤ 500. Sexual behavior and length of HIV potentially affect the probability of CD4:CD8 < 0.3. Both CD4 and CD4:CD8 ratio must be tested at baseline and follow-up.

Keywords: T $CD4^+$, CD4:CD8, prevalence ratio, syphilis, HIV, probability

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a single-stranded ribonucleic acid (RNA) lentivirus, which causes HIV infection/Acquired Immunodeficiency Syndrome (AIDS).[1,2] In 2015 there were 36.7 million people with HIV/AIDS worldwide. There were 2,270 HIV cases in Central Java in 2017 dominated by male and 25-49 years old group. There were 1,409 AIDS cases in Central Java in 2017 with 66.36% males and 72.96% aged 25-49 years old with the highest mortality in this age group. In Surakarta, it was reported that there were 118 new HIV cases in 2017 comprised of 57 HIV and 61 AIDS. A higher number of the male were affected by HIV than the female due to the increase of Male Sex Male (MSM) and Female Sex Worker (FSW) users with the mortality found in 7 males and 2 females.[3,4] The service for sexually transmitted and HIV infections in FSWs in Surakarta was limited and focused more on HIV infection.[5]

Treponema pallidum, the bacterium causing syphilis is transmitted via sexual intercourse, maternal to fetal, and blood transfusion.[6,7] Syphilis population reached 12 million people in 1999 worldwide and more than 90% of cases were found in developing countries. There were 181 syphilis cases in 2017 in Central Java which occurred mostly in people aged 25-49 years old (59.67%) and male (60.77%).[3] Syphilis was found predominantly in HIV patients, especially those who did MSM.[8]

Syphilis genital ulcers increase HIV transmission and infection. Simple columnar epithelium of anal mucosa is susceptible to damage, thereby facilitating HIV infection to lamina propria which is rich in lymphocytes. The risk of HIV transmission through receptive anal intercourse is higher than that of vaginal.[9] Higher HIV viral load and lower CD4 are caused by further T $CD4^+$ depletion, failure of both HIV and syphilis therapies, and development to neurosyphilis had been reported in HIV-syphilis co-infection. HIV-syphilis co-infection causes more

difficulties in establishing syphilis diagnosis and worse clinical features of syphilis.[10,11] These difficulties and complications commonly occur in high-risk sexual behavior and HIV-syphilis coinfection.[1]

The immune system of HIV patients is influenced by many factors. Demographic factors (age, gender, sexual behavior, education, occupation, marriage, income, proximity to health facilities), clinical factors (risk, initial clinical stage, baseline lymphocyte and T $CD4^+$ count, other infections), and treatment factors (regimen, compliance, length of treatment) are considered to affect the immune response of HIV patients.[12-14]

Gender is thought to influence the immunity. In healthy population, male had higher CD8 ($p>0.05$) and CD8 percentage (%CD8) ($p<0.01$) than female.[15] leukocyte count, T $CD4^+$ count, % T $CD4^+$ and CD4:CD8 ratio in female were higher than male. General immune activation, inflammation activation, innate immune response and their interferon-alpha level in female were also found higher than male. Females have a 40% lower HIV viral load, despite their faster progression to AIDS compared to males with the same viremia levels. A study by Prasetyo and Zaini reported that the differences in CD4 counts in healthy population between male and female were not significant and healthy males often had CD4 count less than 500 cells/μL, as it is related with testosterone, androgen and glucocorticoid.[9,15,16]

Age is one of HIV transmission risk factors. People age between 20 and 39 years old are sexually active. Elderly people with HIV have a higher risk of worsening to AIDS-associated with immunosenescent, thymus involution, slower therapeutic response and post anti-retroviral (ARV) reconstitution, higher expression of the chemokine receptor, lower production of interleukin (IL)-2 and its receptors with more intolerance to side effects of ARV toxicity.[9,16-17] In addition to a study by Prasetyo and Zaini, a study by Yusra *et al.*, showed similar findings that older age groups had a greater risk of T $CD4^+$ < 500 cells/μL, while Uppal *et al.* suggested that differences in immunity were not significant among age groups.[5-17]

T $CD4^+$ cell count and identification of other diseases/opportunistic infections must be carried out for the classification of immunodeficiency and prophylaxis for opportunistic infection. The anti-retroviral drug is immediately administered to people with HIV/AIDS (PLWHA) without considering clinical stadium and T $CD4^+$ count. Patients who started receiving ARV with the T $CD4^+$ of >500 cells/μL had a lower risk of worsened progression of AIDS/non-AIDS compared to those with T $CD4^+$ ≥ 300 cells/μL. Insignificant, mild, advanced and severe immunosuppression status can be presented as T $CD4^+$ > 500 cells/μL, 350-499 cells/μL, 200-349 cells/μL, and < 200 cells/μL, respectively.[18,19] After ARV treatment, the T $CD4^+$ count should increase 100-140 cells/μL per year with an acceleration response in the first 3 months.[7] Approximately 10% of HIV patients with ARV developed to AIDS in less than five years.[9] A five-year fatality rate of AIDS was 100%.[20] Depletion of T $CD4^+$ count was accompanied by increased T $CD8^+$, leading to inverted CD4:CD8 ratio.[21]

People with HIV/AIDS who started ARV with CD4:CD8 > 0.5 have greater possibility of its normalization.[22] Non-AIDS events in PLWHA with CD4:CD8 < 0.3 were 2 times higher than those with CD4:CD8 0.3-0.45 or > 0.45.[23]

Studies about the effects of age, gender, syphilis infection, sexual behavior, length of HIV, and length of ARV on the immunity status of the HIV population with syphilis remain unclear with various results. This study aimed to determine the association of syphilis infection and other risk factors with the immunity of HIV patients on ARV.

METHODS

A cross-sectional study was performed from April 24th to June 20th, 2019 with the permission of the Ethics Committee of Dr. Moewardi General Hospital Surakarta with number 544/IV/HREC/2019. Purposive sampling involved subjects from VCT in Dr. Moewardi General Hospital (DMGH) aged ≥ 18 years old.

This research involved 74 patients with HIV aged ≥ 18 years old who routinely took ARV from VCT in DMGH. All of them had assigned informed consent. They had been examined for syphilis using SD Syphilis 3.0 (Multi) Bioline and Venereal Disease Research Laboratory (VDRL) Plasmatec. FACSCanto II flow cytometer was used for CD4 count and CD4:CD8 ratio tests. The exclusion criteria were subjects with lipemic, hemolysis, and icteric samples for syphilis test; clotting formation in tubes with ethylenediaminetetraacetic acid (EDTA); incomplete data (age, gender, sexual behavior, baseline CD4, length of HIV and length of ARV) and false positive or false negative results of syphilis test which needed confirmation. There was no lipemic, hemolysis, and icteric samples. Sample with any different results of syphilis examinations (Bioline or Plasmatec) was excluded to avoid false positive or false negative of syphilis test results. All tests were carried out in the Clinical Pathology Installation of DMGH Surakarta.

Statistical analysis was performed with SPSS 21. Mann-Whitney and T-test were used to determine the differences in characteristic data. Dependent and independent variables have been categorized into two categories. Categorical data were analyzed using bivariate analysis of the Chi-Square test. All variables with p-value <0.25 were analyzed using backward multivariate logistic regression and a p-value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Precision test results of T CD4$^+$ and CD4:CD8 with FACSCanto II using human blood from the healthy donor (both syphilis and HIV non-reactive) showed that difference between tests was ≤ 50 cells/μL and coefficient of variation was <10% as recommended by The United States Department of Health and Human Services and protocol of World Health Organization, indicating that BD FACSCanto II instrument was proper to be used. Precision, minimum value, maximum value, and CV of T CD4$^+$ count were 888.56±3.979, 883.17, 892.25, and 0.45, respectively. In addition, precision, minimum value, maximum value, and CV of CD4:CD8 were 0.988±0.051, 0.94, 1.07, and 5.13, respectively.[24,25]

Most of the subjects in this study were senior high school graduates (Table 1). One research showed that low educational background followed by a lack of knowledge had significant effects on HIV/AIDS incidence.[20] Another study showed that education background was not related to the risk of immunological failure.[26] The immune response of HIV patients were influenced by demographic, clinical, and treatment factors.[17]

Table 1. Characteristic data of subjects based on T CD4$^+$ count and CD4:CD8 ratio

Parameter	Total n=74 (100%)	T CD4$^+$ Count		P	CD4:CD8		P
		≤ 500 (n=46)	>500 (n=28)		< 0.3 (n=27)	≥ 0.3 (n=47)	
Education							
Elementary	n=9 (12.16%)	6 (13.04%)	3 (10.71%)		6 (22.22%)	3 (6.38%)	
Junior HS	10 (13.51%)	7 (15.22%)	3 (10.71%)		4 (14.81%)	6 (12.77%)	
Senior HS/equal	44 (59.46%)	29 (63.05%)	15 (53.58%)		13 (48.15%)	31 (65.96%)	
Diploma	6 (8.11%)	3 (6.52%)	3 (10.71%)		3 (11.12%)	3 (6.38%)	
Bachelor	5 (6.76%)	1 (2.17%)	4 (14.29%)		1 (3.70%)	4 (8.51%)	
Occupation							
Entrepreneur	6 (8.11%)	3 (6.52%)	3 (10.71%)		0 (0%)	6 (12.77%)	
Private sector	34 (45.95%)	20 (43.48%)	14 (50%)		8 (29.63%)	26 (55.31%)	
Employee	11 (14.86%)	9 (19.57%)	2 (7.15%)		7 (25.93%)	4 (8.51%)	
Labor	4 (5.41%)	1 (2.17%)	3 (10.71%)		1 (3.7%)	3 (6.38%)	
Merchant	2 (2.70%)	1 (2.17%)	1 (3.57%)		1 (3.7%)	1 (2.13%)	
Farmer	2 (2.70%)	2 (4.35%)	0 (0%)		1 (3.7%)	1 (2.13%)	
Housewife	13 (17.57%)	8 (17.39%)	5 (17.86%)		7 (25.93%)	6 (12.77%)	
Scholars	2 (2.70%)	2 (4.35%)	0 (0%)		2 (7.41%)	0 (0%)	
Marriage							
No	17 (22.97%)	12 (26.09%)	5 (17.86%)		11 (40.74%)	6 (12.77%)	
Yes	57 (77.03%)	34 (73.91%)	23 (82.14%)		16 (59.26%)	41 (87.23%)	
%CD4 (%)	18.5± 8.2[1]	14,3± 6.6[1]	25.4±5.6[1]	0.0001[1]	10.3±4.4[1]	23.2±5.9[1]	0.0001[1]
Baseline T CD4$^+$ (cells/uL)	44.37 (31.2-63.1)[1]	31.54 (24.6-48.7)[1]	138 (3.0-641.8)[2]	0.005[2]	34.8 (19.3-62.8)[1]	66 (3.0-641.8)[2]	0.138[2]
Delta T CD4$^+$ (cells/uL)	336.2±235.5[1]	196.3±143.7[1]	566.1±165.6[1]	0.0001[1]	145.9±142.4[1]	445.5±207.6[1]	0.0001[1]
T CD8$^+$ (cells/uL)	999.08 (899-1,111)[1]				497.16 (371-667)[1]	985.37 (887-1,095)[1]	0.38[1]

Note: HS: High School CD: Cluster of Differentiation; [1]mean±SD, geometric mean (95% confidence interval), T-test, data display of normally or abnormally distributed data; [2]median (minimum-maximum), Mann-Whitney test, data display of abnormally distributed data; [3]Chi-Square or Fisher' exact test; n: number

Table 2. Difference tests between variables

Variable	Total 74 (100%)	T CD4+		P	CD4:CD8		P
		≤500 (n=46)	>500 (n=28)		<0.3 (n=27)	≥0.3 (n=47)	
Gender				0.016[3]			0.074[3]
Male	37 (50%)	28 (60.87%)	9 (32.14%)		17 (62.96%)	20 (42.55%)	
Female	37 (50%)	18 (39.13%)	19 (67.86%)		10 (37.04%)	27 (57.45%)	
Age	39.74±9.69[1]	40.55±10.03[1]	38.40±9.13[1]	0.279[1]	37.71 (33.4-42.6)[1]	39.90±8.09[1]	0.298[1]
> 42 y.o	28 (37.84%)	20 (43.48%)	8 (28.57%)		11 (40.74%)	17 (36.17%)	
18-42 y.o	46 (62.16%)	26 (56.52%)	20 (71.43%)		16 (59.26%)	30 (63.83%)	
Sexual behavior				0.500[3]			0.007[3]
Anal	17 (22.97%)	11 (23.91%)	6 (21.43%)		11 (40.74%)	6 (12.77%)	
Vaginal	57 (77.03%)	35 (76.09%)	22 (78.57%)		16 (59.26%)	41 (87.23%)	
Syphilis infection				0.497[3]			0.229[3]
Yes	12 (16.22%)	8 (17.39%)	4 (14.29%)		6 (22.22%)	6 (12.77%)	
No	62 (83.78%)	38 (82.61%)	24 (85.71%)		21 (77.78%)	41 (87.23%)	
Length of HIV (years)	4.18 (0.63-11.25)[2]	2.94 (2.32-3.73)[1]	5.95±2.68[1]	0.005[1]	2.0 (1.49-2.69)[1]	5.83±2.58[1]	
>5 years	33 (44.59%)	31 (67.39%)	18 (64.29%)		4 (14.81%)	29 (61.70%)	0.0001[1]
0-5 years	41 (55.41%)	15 (32.61%)	10 (35.71%)		23 (85.19%)	18 (38.30 %)	
Length of ARV	4.09 (0.61-11.25)[2]	2.81 (2.22-3.57)[1]	5.71 ± 2.55[1]	0.0001[1]	1.87 (1.42-2.48)[1]	5.71±2.55[1]	
0-5 years	41 (55.41%)	11 (23.91%)	10 (35.71%)		23 (85.19%)	18 (38.30%)	0.0001[1]
>5 years	33(44.59%)	35 (76.09%)	18 (64.29%)		4 (14.81%)	29 (61.70%)	
T CD4+count	444.32±259.86[1]	277.27±130.01[1]	718.75±169.59[1]	0.0001[1]	197 (13.17 618)[2]	564.46±232.51[1]	0.0001[2]
>500/uL	28 (37.84%)				2 (7.41%)	26 (55.31%)	
≤500/uL	46(62.16%)				25 (92.59%)	21 (44.69 %)	
CD4:CD8 ratio	0.433±0.245[1]	0.32±0.17[1]	0.62±0.23[1]	0.0001[1]	0.21 (0.03-0.29)[2]	0.54 (0.49-0.59)[2]	0.0001[2]

Note: [1]mean±SD, geometric mean (95% confidence interval), T-test, data display of normally or abnormally distributed data; [2]median (minimum-maximum), Mann-Whitney test, data display of abnormally distributed data; [3]Chi-Square or Fisher' exact test; n: number; HIV: Human Immunodeficiency Virus; CD: Cluster of Differentiation

Most of the subjects worked with the private sector. Some researchers found that PLWHA who settle indeed lack of stress, risk or work pressure, leading to better immune status and vice versa.[27-29] Another study showed that employment was not related to the immunological failure of PLWHA.[26] Stress and financial condition were able to promote risky sexual behavior.[30]

The number of married subjects was greater than unmarried ones. This was in accordance with the studies by Saktina and Bagus.[28] Another study reported that the lowest incidence of HIV was found in the married subjects who live together compared to those who live together with their sexual partners, single, widowed and divorced.[31]

The T $CD4^+$ ≤ 500 cells/μL group was dominated by male subjects, while the T $CD4^+$ > 500 cells/μL group was dominated by female subjects (p=0.074) (Table 2). This was in accordance with a study by Klatt and Iswara *et al*.[9,26] However, different results were found in the study by Adiningsih *et al*. They reported that females with HIV had a higher risk of immunodeficiency than males.[27] Prasetyo and Zaini suggested that the difference of T $CD4^+$ count between male and female in healthy populations was not significant.[16] Susilowati *et al*. revealed that there was no relationship between gender and the incidence of HIV/AIDS.[20] Olsen and Kovacs in 2011 showed that there was an association between the hypogonadal condition and increased thymic output of T-cells thus increasing recent thymic emigrants in peripheral blood that will be reversed by androgen replacement.[32] The CCR5 expression modulated by sex hormones is still controversial. Study of HIV-1–seronegative individuals showed that the density but not the percentage of $CCR5^+CD4^+$ T-cells was lower in females than in males. The CCR5 acts as HIV-1 coreceptor to enter and infect target cells such as T $CD4^+$.[33] This study found that age, sexual behavior, and syphilis infection were insignificantly associated with T $CD4^+$ (Table 3).

The prevalence of T $CD4^+$ cell ≤ 500 cells/μL in subjects with HIV > 5 years was (3/5) times significantly lower than subjects with 0-5 years of HIV infection. Su *et al*. suggested that the interval between HIV diagnosis and the initiation of ARV had bad effects on adherence and outcome. Every 100 days of extensive pre-treatment waiting time increased loss to follow-up by 20% and mortality rate by 11%.[34] This study population was HIV patients on ARV. The median duration gap between HIV diagnosis and start of ARV treatment was 14.6 days

Table 3. Cross tabulation of categorical variables

Variable	T $CD4^+$		PR (95% CI)	p	CD4:CD8		PR (95% CI)	p
	≤500	>500			<0.3	≥ 0.3		
Gender			1.556	0.016*			1.700	0.074
Male	28	9	(1.066-2.270)		17	20	(0.901-3.206)	
Female	18	19			10	27		
Age (years)			1.264	0.151			1.129	0.444
>42	19	8	(0.895-1.785)		10	17	(0.615-2.073)	
18-42	27	20			17	30		
Sexual behavior			1.054	0.500			2.305	0.007*
Anal	11	6	(0.701-1.583)		11	6	(1.338-3.972)	
Vaginal	35	22			16	41		
Syphilis infection			1.088	0.497			1.476	0.229
Yes	8	4	(0.696-1.700)		6	6	(0.760-2.868)	
No	38	24			21	41		
Length of HIV (years)			0.601	0.008*			0.216	0.0001*
>5	31	18	(0.398-0.908)		4	29	(0.083-0.563)	
0-5	15	10			23	18		
Length of ARV (years)			1.663	0.008*			4.628	0.0001*
0-5	11	10	(1.101-2.512)		23	18	(1.776-12.060)	
>5	35	18			4	29		

*p-value<0.05 significant, HIV: Human Immunodeficiency Virus, CD: Cluster of Differentiation, ARV: anti-retroviral, PR: Prevalence Ratio, CI: Confidence Interval

and the modus was 0 day (HIV diagnosis and the start of ARV were in the same day), indicating that length HIV infection is in line with a length of ARV, that more viruses were eradicated, leading to the elevated T CD4$^+$ count and the lower prevalence of T CD4$^+$ ≤500 cells/μL.

The prevalence of CD4 cell count of ≤500 cells/μL in HIV subjects with ARV 0-5 years was 1.66 times significantly higher than subjects with ARV > 5 years. This was consistent with the study by Adiningsih *et al*.[27] Activation of T-cells (co-expression of CD38 and HLA-DR on the T-cell surface) and T-cell exhaustion (expression of programmed cell death 1 (PD-1)) occurred after four years of ARV therapy. Activation and exhaustion of CD4$^+$ and CD8$^+$ T-cells were significantly higher in patients with suboptimal CD4$^+$ T-cells reconstitution compared to optimal and superoptimal responders ($p<0.05$). Increased apoptosis and intrinsic T-cell death play a role in incomplete recovery of CD4$^+$ T-cells count. Programmed cell death-1 expression is increased during HIV-1 infection and negatively regulates T-cell activity. There are conflicting data on the influence of T-cell activation on CD4$^+$ T-cell recovery among patients with successful ARV.[35] Other literature said that the CD4$^+$ T-cell amount still increased in subjects with ARV for 5-7 years. CD4$^+$ T count below the normal range despite ARV therapy showed insufficient suppression of HIV replication and/or start of ARVs in older age.[36]

This research findings gender, age, and syphilis infection were insignificantly associated with a CD4:CD8 ratio. The difference in CD4:CD8 ratio between acute and chronic HIV infection with/without syphilis was not significant.[8] The likelihood of CD4:CD8 ratio normalization after 72 weeks of ARV in HIV alone was greater than HIV-syphilis co-infection.[37]

The prevalence of CD4:CD8 ratio < 0.3 in HIV subjects with anal sexual behavior was 2.305 times significantly higher than those with vaginal sexual behavior. The gastrointestinal mucosa is uniquely susceptible to HIV-1 infection and supportive of HIV-1 replication. The T CD4$^+$ lymphocytes in a gastrointestinal mucosal express a greater percentage of CCR5 chemokine coreceptors than those in circulation with the same expression level of CXCR4 chemokine coreceptor. One study reported that both the CD4$^+$ T-cell percentage and CD4:CD8 ratio in the mucosa of subjects with primary HIV infection was significantly lower compared to those in peripheral blood.[38]

Activation of T-cells and HIV replication in the anal and rectal mucosa due to the loss of specific T CD4$^+$ lymphocytes clones in the gastrointestinal tract accelerates the immunosenescent process. The anorectal epithelium shows the highest probability of HIV-transmission than oral and genital. The gastrointestinal tract is the main source of lymphocytes in the body with a predominance of CD4$^+$ T-cells that express CCR5. The anal mucosal epithelium is susceptible to damage during anal intercourse resulting in increased transmission and HIV infection of CD4 in lamina propria. Cervico-vaginal fluids contain vaginal transudate, mucus, antimicrobial factors, chemokines, and cytokines (defensins, SLPI, Elafin, RANTES, and CCL2) that have been associated with protection against HIV infection. Ectocervical and vaginal mucosae also consist of the multi-stratified epithelium with tight junctions in the deepest monolayers of cells. The inner foreskin has a higher frequency of HIV target cells such as T CD4$^+$ lymphocytes, Langerhans, macrophages, and DCs.

Mucosal foreskin epithelium in circumcised male is removed, thus leaving a dry keratinized epithelial surface which is more resistant to HIV infection as demonstrated in-vitro and in-vivo.[9,38,39]

The prevalence of CD4:CD8 ratio < 0.3 in patients with a length of HIV infection > 5 years (1/5) times was significantly lower than that of 0-5 years. The CD4$^+$ T-cells percentage continued to decline in chronic HIV-infected subjects without ARV. Normalization of CD4:CD8 ratio to 2.9 occurred after complete ARV for two years. Patients on immediate and complete ARV therapy reducing the interval between the diagnosis and the initiation of ARV and increasing the possibility of normalization of CD4:CD8 ratio.[38]

The prevalence of CD4:CD8 ratio < 0.3 in subjects with ARV for 0-5 years was 4.628 times significantly higher than that of > 5 years. The probability of normalization ratio after 5 years of ARV occurred in 6.1%; 21%; 16% and 4.5% subjects with baseline CD4$^+$ T-cells < 200, baseline CD4$^+$ T-cells > 350, baseline CD8$^+$ T-cells < 500 and baseline CD8$^+$ T-cells >1150 cells/μL. The baseline CD4$^+$ T-cells, time-updated HIV RNA suppression, and sexual relations other than partners were thought to have contributed to its normalization.[21] Saracino *et al*. proved that 37% of the subjects achieved normal CD4:CD8 ratio ≥ 0.9 (median 0.42; baseline 0.16) with median 16 years of ARV treatment and the progression of CD4:CD8 ratio without plateau phase with significant differences at baseline CD4:CD8,

baseline % $CD4^+$ T-cells, $CD4^+$ T-cells, % $CD4^+$ T-cells, $CD8^+$ T-cells and % $CD8^+$ T-cells.[40] Mussini *et al*. suggested that 12% of subjects achieved normal ratio within two years.[23] Thornhill *et al*. reported that the subjects with a wider interval between seroconversion and the initiation of ARV had less probability to achieve ratio normalization.[41] The $CD4^+$ T-cells count and baseline $CD4^+$ T-cells were significantly lower in the CD4:CD8 < 0.3 group. The prevalence of $CD4^+$ T-cells ≤ 500 cells/μL in subjects with an ARV duration of 0-5 years in this study proved to be significantly 3.72 times higher than that of > 5 years.

Multivariate analysis showed that age, gender, and the length of HIV infection were significantly associated with $CD4^+$ T-cells, whereas the duration of HIV and sexual behavior were significantly associated with CD4:CD8 ratio. Some literatures suggested that probability can be calculated using equation of p = 1 / [1 + e x p (- y)], p = p r o b a b i l i t y, y=constanta+B1(x1)+B2(x2)+etc. The values of constanta, B1, B2 from B column of SPSS 21, table were input as variables in the equation table. Value x1, x2, etc. should be filled by one or zero.[42,43] The probability of $CD4^+$ T-cells ≤500 cells/μL can be calculated with the formula y=0.185+1.181 (gender)+1.239 (age)-1.304 (duration of HIV). The probability of $CD4^+$ T-cells ≤ 500 cells/μL in subjects aged > 42 y.o. on ARV was 80.60%. The probability of $CD4^+$ T-cells ≤ 500 cells/μL in male subjects was 79.67%. The probability of $CD4^+$ T-cells ≤ 500 cells/μL in male subjects aged > 42 y.o. was 93.12%. The probability of $CD4^+$ T-cells ≤ 500 cells/μL in male subjects aged > 42 y.o. with the duration of HIV > 5 years was 78.60%. The probability of CD4:CD8 < 0.3 can be calculated with formula p=1/[1+exp(-y)], p=probability, y=konstanta+1,274 (sexual behavior)-2,083 (duration of HIV). The probability of CD4:CD8 < 0.3 in subjects with anal sexual behavior was 75.93%, whereas the probability of CD4:CD8 < 0.3 in subjects with anal sexual behavior and duration of HIV > 5 years was 28.21%.

Table 4. Logistic regression analysis of variables with T $CD4^+$

Variable	PR	95% CI	P
Model 1			
Gender	3.256	1.07-9.91	0.038*
Age	3.451	1.01-11.74	0.047*
Length of HIV	0.271	0.09-0.82	0.020*

*p <0.05: significant, PR: Prevalence Ratio, HIV: Human Immunodeficiency Virus; CI: Confidence Interval; CD: Cluster of Differentiation

Table 5. Logistic regression analysis of variables with CD4:CD8 ratio

Variable	PR	CI 95%	P
1st model			
Sexual behavior	3.575	1.006-12.705	0.049*
length of HIV	0.125	0.036-0.431	0.001*
2nd model			
Gender	0.698	0.184-2.655	0.598
Sexual behavior	4.437	0.977-20.150	0.054
length of HIV	0.116	0.033-0.415	0.001*
3rd model			
Gender	0.686	0.178-2.644	0.584
Sexual behavior	4.193	0.824-21.342	0.084
Syphilis infection	1.177	0.201-6.895	0.857
length of HIV	0.115	0.032-0.414	0.001*

*p<0.05: significant, CI: Confidence Interval, CD: Cluster of Differentiation, PR: Prevalence Ratio, HIV: Human Immunodeficiency Virus

Cross-sectional design in this study may yield relationship among variables remained unclear. Better and clear relationship in next research will be achieved by adding adherence, hormonal effects, type and duration of antibiotic usage, frequency of changes in ARV regiment, differences in HIV-ARV duration and delta $CD4^+$ T-cells in analysis and using case control or cohort design.

CONCLUSION AND SUGGESTION

There was no significant association between syphilis infection and the immunity of HIV patients on ARV based on T $CD4^+$ count and CD4:CD8 ratio. The length of HIV, gender, and age was significantly associated with T $CD4^+$ count, while the length of HIV and sexual behavior were significantly associated with CD4:CD8 ratio. Age, gender, and length of HIV potentially affected the probability of T $CD4^+ \leq 500$. Sexual behavior and duration of HIV potentially affected the probability of CD4:CD8 < 0.3. It was recommended to measure $CD4^+$ T-cells count and CD4:CD8 ratio at baseline and follow-up.

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