

ESTABLISHING MEAN CD4+ T CELL VALUES AMONG HEALTHY JAVANESE ADULTS IN INDONESIA

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Abstract. The objective of this study was to establish mean CD4+ T cell values among healthy Javanese adults in Indonesia. Two hundred forty-one healthy adults (119 women and 122 men), aged 18-65 years, were enrolled in the study. CD4+ T cells were analyzed by immunophenotyping. The mean absolute CD4+ T cell count was 753.3 ± 270.3 cells/ μ l (median= 725.0 cells/ μ l) and the mean CD4+ T cell percentage was $32.6 \pm 7.7\%$, (median= 31.0%). Women had a slightly higher mean absolute CD4+ T cell count and CD4+ T cell percentage (779.1 ± 271.0 cells/ μ l; $33.4 \pm 8.2\%$) than men (728.2 ± 268.3 cells/ μ l; $31.8 \pm 7.1\%$), but the differences were not statistically significant ($p=0.126$, $p=0.216$, respectively). The mean absolute CD4+ T cell varied significantly by age group ($p=0.002$). Sixty-one point seven percent of men studied (37/60) had a CD4+ T cell count less than 500 cells/ μ l (OR 1.8; 95%CI=1.001-3.300). Absolute CD4+ T cell counts among Javanese Indonesians varied significantly by age.

Keywords: CD4+ T cells, reference value, Javanese, Indonesia

INTRODUCTION

HIV infection has become a worldwide epidemic (UNAIDS, 2014) and an epidemic in Indonesia (Ministry of Health, 2014). In September 2014, 150,296 people were reported to be living with HIV in Indonesia (Ministry of Health, 2014). These people with HIV and a low CD4+ T cell count require antiretroviral (ART) therapy to prevent morbidity and

mortality. Among HIV-infected persons, monitoring of ART therapy and disease progression relies on the CD4+ T cell count and viral load (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2014). Absolute CD4+ T cell counts and percentages are markers of immune function and are used to determine initiation of ART (Hulgan *et al*, 2007; Panel on Antiretroviral Guidelines for Adults and Adolescents, 2014).

The Indonesian Ministry of Health's guidelines for ART therapy in HIV infection recommends initiation of therapy when the patient's absolute CD4+ T cell count decreases to less than 350 cells/ μ l to improve the survival rates and reduce HIV-related disease progression (Ministry of Health, 2011; Panel on Antiretroviral Guidelines for Adults and Adolescents,

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2014). The absolute CD4+ T cell count and percentage may be influenced by age, gender and race (Mair *et al*, 2008; Garcia-Dabrio *et al*, 2012; Moreno-Galván and Palafox, 2013; Torres *et al*, 2013). Investigation of a normal CD4+ T cell count in a healthy population will help ensure HIV-infected patients receive proper management. Given the importance of CD4+ T cell counts in the management of HIV, each country should have its own absolute CD4+ T cell count and percentage reference values (Sagnia *et al*, 2011); in Indonesia this has not yet been determined to the best of our knowledge. The present study was conducted to establish the mean absolute CD4+ T cell count and percentage reference values for healthy Javanese adults in Indonesia as a pilot study for establishing mean reference CD4+ T cell counts and percentages among healthy adult Indonesians.

MATERIALS AND METHODS

Study population

Since 2009, our research group has performed a molecular epidemiology study of human blood borne viruses by collecting epidemiological data and blood samples from high risk communities, such as drug abusing inmates in prisons (Prasetyo *et al*, 2013) and jails, men having sex with men in Surakarta (Prasetyo *et al*, 2014) and the surrounding cities, commercial sex workers, injecting drug users and heterosexual contact with an HIV+ partner in Central Java, Indonesia. During 2010-2012, healthy Javanese Indonesian adults living in high risk areas in Surakarta, Sragen, Klaten, Boyolali, Magelang and Semarang were invited to participate in this study. Participants were interviewed using a structured questionnaire to obtain information about lifestyle (smoking, al-

cohol consumption, stress level and daily sleep), pregnancy, vaccinations during the previous 6 months, infections during the previous 4 weeks, use of antibiotics during the previous 4 weeks, hospitalizations during the previous 2 years, medication use, and history of current or recent comorbid conditions. The participants were also asked about symptoms, such as fever, cough and weight loss, to rule out current or recent infections. Subjects who gave a positive history for any of these items were excluded from the study. Ethical approval for the study was obtained from the institutional ethics committee review boards of the Faculty of Medicine, Sebelas Maret University and Dr Moewardi General Hospital, Surakarta, Indonesia. Written informed consent was obtained from each participant prior to being included in the study. The study was conducted according to the principles of the Declaration of Helsinki (WMA, 2008).

Blood sample collection and screening for infections

Blood samples were obtained from each participant in the morning between 9:00 AM to 12:00 noon, and placed in ethylene diamine-tetra-acetic acid (EDTA) tubes, kept at room temperature and analyzed the same day. The blood samples were screened for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), human T lymphotropic virus-1/2 (HTLV-1/2), torque teno virus (TTV) and *Toxoplasma gondii* by serological and/or molecular assays, as described previously (Prasetyo *et al*, 2013; 2014). Syphilis testing was also performed with the SD Bioline Syphilis test, 3.0 (Standard Diagnostics, Suwon City, South Korea). All assays were performed according to the manufacturer's instructions. All samples were tested in duplicate.

CD4+ T cells counts

CD4+ T cell subpopulations were analyzed using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). In brief, 20 μl of Tritest (CD3/CD4/CD45 - Becton Dickinson Immunocytometry Systems) monoclonal antibodies were added to a Trucount tube containing reference beads (Becton Dickinson Immunocytometry Systems). Whole blood (50 μl) was mixed and incubated at room temperature (20°C-25°C) for 15 minutes in the dark. Red blood cells were then lysed by adding 450 μl of fluorescence-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems). The tubes were then incubated at room temperature for 15 minutes and the blood analyzed within six hours.

Statistical analysis

Means, standard deviations, medians and odds ratios (OR) with 95% confidence intervals (CI) for absolute CD4+ T cell counts and percentages were calculated using SPSS, version 21 (IBM, Armonk, NY). Comparison of mean values between variables was performed with the Mann-Whitney and Kruskal-Wallis tests.

RESULTS

During 2010-2012, 241 (119 women and 122 men) healthy Javanese Indonesian adults aged 18-65 years were enrolled in the study. All subjects were negative for HIV on serological and molecular assays and negative for HBV, HCV, HDV, HTLV-1/2, TTV, *Toxoplasma gondii* and syphilis disease by serological and/or molecular assays. The health of all the participants was reported as good.

The median absolute CD4+ T cell count was 725.0 cells/ μl (780 cells/ μl for women and 683 cells/ μl for men). The

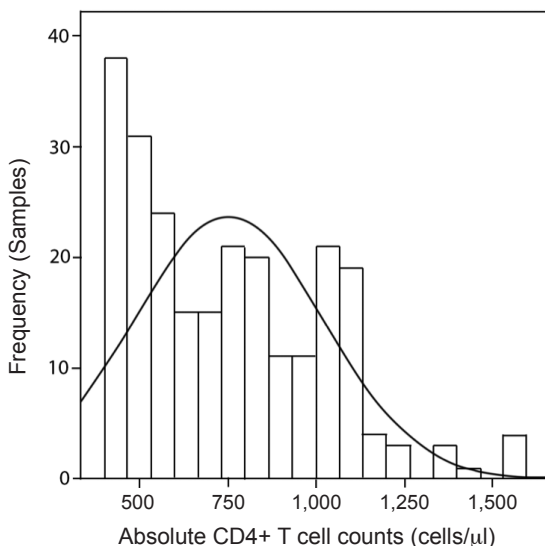


Fig 1–Distribution of absolute CD4+ T cell counts in the study population.

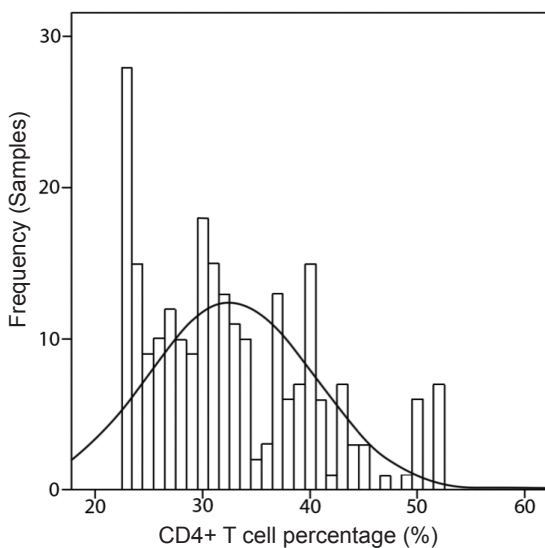


Fig 2–Distribution of CD4+ T cell percentage in the study population.

median CD4+ T cell percentage was 31.0% (32.0% for women and 31.0% for men). The overall mean absolute CD4+ T cell count was 753.3 ± 270.3 cells/ μl (Fig 1, 2). The absolute CD4+ T cell counts varied significantly by age group ($p=0.002$).

Table 1
Absolute CD4+ T cell counts and percentages by gender and age.

Gender and age groups	% (number)	Absolute CD4+ T cell count \pm SD in cells/ μ l	CD4+ T cell percentage \pm SD in percent
Women			
18-20	16.8 (20/119)	870.9 \pm 245.6 ^a	30.8 \pm 5.9
21-25	16.8 (20/119)	708.6 \pm 285.2	27.9 \pm 6.5 ^b
26-30	16.8 (20/119)	700.0 \pm 283.5 ^a	33.4 \pm 7.7
31-35	16.8 (20/119)	736.2 \pm 175.9 ^a	34.7 \pm 6.8
36-40	16.8 (20/119)	835.1 \pm 217.9	38.7 \pm 8.7 ^b
41-65	16.0 (19/119)	826.3 \pm 366.7 ^b	34.9 \pm 9.6
Men			
18-20	16.4 (20/122)	856.9 \pm 241.8 ^a	29.2 \pm 4.7
21-25	16.4 (20/122)	742.6 \pm 299.8	31.4 \pm 5.3 ^b
26-30	16.4 (20/122)	630.1 \pm 207.7 ^a	33.3 \pm 7.4
31-35	16.4 (20/122)	818.5 \pm 288.2 ^a	29.7 \pm 5.9
36-40	16.4 (20/122)	754.4 \pm 236.8 ^a	32.7 \pm 6.7 ^b
41-65	18.0 (22/122)	581.1 \pm 243.6 ^{a,b}	34.3 \pm 10.2

^a $p < 0.05$ for difference by age group.

^b $p < 0.05$ for difference by gender.

Those aged 18-20 years had significantly higher absolute CD4+ T cell counts than the older age groups (Table 1).

The women in our study had a slightly higher mean absolute CD4+ T cell count (779.1 \pm 271.0 cells/ μ l) than the men (728.2 \pm 268.3 cells/ μ l) but the difference was not significant ($p=0.126$). The mean absolute CD4+ T cell among women aged 18-20 years was significantly higher than among women aged 26-30 and 31-35 years ($p=0.025$, $p=0.021$, respectively). The mean absolute CD4+ T cell count among men aged 18-20 years was significantly higher than among men aged 26-30 and 41-65 years ($p=0.03$, $p=0.000$, respectively). An absolute CD4+ T cell count less than 500 cells/ μ l was found in 19.3% of women (23/119) and 30.3% of men (37/122); having a lower count was more likely among men (61.7%, 37/60, OR=1.8; 95%CI: 1.001-3.300) than women, 32.4% (12/37) aged 41-65 years.

The mean CD4+ T cell percentage for all subjects was 32.6 \pm 7.7%. The mean CD4+ T cell percentage among women (33.4 \pm 8.2 %) was slightly higher than the men (31.8 \pm 7.1 %) but the difference was not significant ($p=0.216$); there was also no significant differences ($p=0.351$) by age group. In the 21-25 year old age group, women had a significantly lower CD4+ T cell percentage than men ($p=0.015$). In the 36-40 year old age group, men had a significantly lower CD4+ T cell percentage than women ($p=0.04$) (Table 1).

DISCUSSION

The absolute CD4+ T cell count and percentage are important data to guide ARV therapy initiation and help in predicting HIV progression to AIDS. In Indonesia, the absolute CD4+ T cell count is a recommended test when deciding on initiation of ARV therapy (Panel on Antiretroviral Guidelines for Adults and

Table 2
Comparison of absolute CD4+ T cell counts and percentages by population.

Country	Absolute CD4+ T cell count ± SD in cells/μl		CD4+ T cell percentages		Reference
	Mean	Median	Mean	Median	
Asians					
Indonesia	753.3 ± 270.3	725.0	32.6 ± 7.7	31.0	
Singapore	838.0 ± 268.0	-	35.6 ± 6.3	-	Chng <i>et al</i> , 2004
Thailand	663.8 ± 223.1	624.0	32.1 ± 5.9	32.0	Assawawitoontip <i>et al</i> , 2003
Hong Kong	760.0 ± 233.0	-	40.3 ± 6.4	-	Wong <i>et al</i> , 2013
China	727.0 ± 255.0	-	-	-	Jiang <i>et al</i> , 2004
South Korea	787.0 ± 290.0	714.0	40.6 ± 8.5	40.0	Choi <i>et al</i> , 2014
Caucasians					
Iran	1,039.9 ± 338.0	-	41.0 ± 7.9	-	Kamallou <i>et al</i> , 2014
Turkey	1,095.0 ± 391.0	1,055.0	47.4 ± 9.1	49.4	Yaman <i>et al</i> , 2005
Oman ^a	1,005.8 ± 319.3	1,805.7	40.4 ± 6.5	35.0	Al-Jabri <i>et al</i> , 2008
Spain	-	-	45.2 ± 7.9	-	Garcia-Dabrio <i>et al</i> , 2012
Brazil	-	844.0	-	39.5	Rudolf-Oliveira <i>et al</i> , 2015
Italy	940.5	885.0	45.1	45.0	Santagostino <i>et al</i> , 1999
Africans					
Mozambique	-	774.0	-	38.7	Tembe <i>et al</i> , 2014
Tanzania	745.9 ± 695.5	722.5	-	-	Ngowi <i>et al</i> , 2009
Nigeria	847.0 ± 307.0	812.0	-	-	Oladepo <i>et al</i> , 2009
Kenya	920	884.0	37.0	37.0	Bosire <i>et al</i> , 2013
Uncertain (Dravida)					
India	919.0 ± 312.0	877.0	38.0	37.0	Thakar <i>et al</i> , 2011

^aHealthy adult males only

Adolescents, 2014; Hulgan *et al*, 2007; Ministry of Health, 2011).

In the present study, the mean and median absolute CD4+ T cell counts among our subjects were 753.3 ± 270.3 cells/μl and 725.0 cells/μl, respectively, similar to China, Hong Kong, and South Korea but lower than among Caucasians (Table 2), suggesting the absolute CD4+ T cell count may be influenced by race or other genetic factors (Ahmadi *et al*, 2001; Chng *et al*, 2004). Women had slightly higher absolute CD4+ T cell counts and percentages in our study than men, but the differences were not significant, con-

sistent with a previous study from Hong Kong (Wong *et al*, 2013). Men were more likely to have an absolute CD4+ T cell count less than 500 cells/μl. The age group most likely to have an absolute CD4+ T cell count less than 500 was 45-65 years old. This could be a result of testosterone or age. Testosterone has a negative effect on thymus function by increasing synthesis and release of local glucocorticoids in the thymus mediated by androgens, causing thymic involution by triggering antiproliferation or apoptosis (Chen *et al*, 2010; Olsen and Kovacs, 2011).

In conclusion, the mean absolute

CD4+ T cell count and CD4+ T cell percentage among our subjects in Indonesia were 753.3 ± 270.3 cells/ μ l (median=725.0 cells/ μ l) and $32.6 \pm 7.7\%$, (median=31.0%), respectively. These values were affected by age and possibly gender.

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