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Research Article

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Investigation of CD4+ T cell numbers in HIV-infected patients among smokers and non-smokers in Thailand

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ABSTRACT

The effects of tobacco smoking on the immunity of HIV-infected patients in the Thai population were studies. The means of absolute CD4+ T lymphocyte count between 22 smoking and 88 non-smoking patients were evaluated and compared using flow cytometry. Results showed that the mean of the absolute CD4+ T lymphocyte count among the HIV-infected smoking group (mean=331 cells/µL) was significantly lower than the HIV-infected non-smoking group (mean=416.19 cells/µL), (P=0.131). The majority of the HIV-infected smokers group still had CD4+ T cell levels lower than 350 cells/µL. Therefore, CD4+ T lymphocytes at the level of 350 cells/µL, according to the treatment guidelines published by WHO, must be maintained. Furthermore, the percentage of CD4+ T lymphocytes in the smoking group was still higher than the non-smoking group.

Keywords: HIV, CD4+ T lymphocytes, Smoking

INTRODUCTION

Thailand has been reported as a high-prevalence area of Human Immunodeficiency Virus (HIV) infection. Recently, a published report in 2011 showed that 34 million people had been suffering from HIV infection [1]. The pathogen's main target is CD4+ T lymphocyte, which plays a crucial role in the immune system by protecting the host from biological invaders. After HIV establishes infection in the host and the virus cannot be suppressed immune system, CD4+ T cell decrease in number together with HIV replication and the host becomes more susceptible to opportunistic infections. Previous surveys found that there was a greater proportion of smokers who were HIV-infected individuals over HIV seronegative [1]. Moreover, there exist many publications indicating the association of smoking with numerous diseases. For example, smoking has been shown to be highly associated with the deterioration of lung function in immunocompetent individuals. Smoking also devastates immune function, which can lead to the development of opportunistic infections such as oral hairy leukemia, bacterial pneumonia and pneumocystis pneumonia. In a female group, smokers have a higher risk of cervical carcinoma when compared to non-smokers. Furthermore, smoking affects the severity of progression and the increased risk of cancers such as lung cancer, oral cancer, throat cancer and Hodgkin's lymphoma [2]. Some reports have illustrated that smoking is related to a higher mortality rate among HIV-infected patients. In addition, smoking is related to skin disease in HIV-infected patients. Smoking HIV seropositive individuals are also at higher risk of coronary heart disease, myocardial infarction (MI), and cardiovascular disease (CVD) when compared to non-smoking HIV patients [2]. Much research has revealed the correlation between a decline in CD4+ T lymphocyte counts and HIV-seropositive smokers [3-5]. The quantity of CD4+ T lymphocyte is highly correlated with disease progression and has been used as a parameter to decide when to start anti-retroviral drugs (ARV) in order to slow AIDS progression. According to a study by Li T et. al, CD4+ T lymphocyte count in a healthy donor group is markedly higher than the HIV-infected group and AIDS patient group [6]. In addition, CD4+ level were influenced by regional factors. Therefore, a study

of the CD4+ T lymphocyte levels between Thai smokers and non-smokers should be conducted to provide information about the CD4+ level to start antiretroviral therapy.

EXPERIMENTAL SECTION

Population and Sample

In this study, we enrolled 110 HIV-infected individuals from King Chulalongkorn Memorial Hospital, Bangkok, Thailand. All patients were categorized into two groups, which were smokers defined by diagnosed as HIV-1 seropositive individuals who have been smoking for at least six months (n=22) and non-smokers (n=88). EDTA whole blood samples were collected using BD Vacutainer (R) Blood Collection Tube (BD Biosciences, USA). CD4+ T lymphocyte determination was performed within six hours after the blood was collected.

T lymphocyte staining and absolute CD4+ T lymphocyte count

For the T-lymphocyte phenotype, EDTA blood specimens were stained with TriTEST; CD3-PerCP, CD4-FITC, CD8-PE (BD Biosciences, USA) following the manufacturer's instructions. Briefly, 50 µL of the sample was incubated at room temperature for 15 minutes in the presence of 20 µL of TriTEST. After incubation, red blood cells were lysed by 100 µL of 1X BD FACS™ lysing solution and incubated at room temperature for 10 minutes. Then, the RBC lysed sample was washed once with Phosphate-Buffer-Saline. Finally, stained samples were resuspended in 200 µL of 0.5% paraformaldehyde. The absolute number of CD4+ T lymphocyte was assessed using BD FACSCalibur™ (BD Biosciences, USA). Helper-T lymphocyte and Cytotoxic-T lymphocyte were defined by CD3+CD4+ and CD3+CD8+ population, respectively. Absolute CD4+ T lymphocyte count was calculated from the percentage of CD4+, percentage of lymphocyte and WBC count provided from the complete blood count (CBC).

Statistical analysis

Absolute CD4+ T lymphocyte counts from both, HIV-1 seropositive smoker and non-smoker groups were collected. The means of the absolute CD4+ T lymphocyte counts between the two groups were compared using t-test (SPSS for Windows version17.0). Four groups of CD4+ T lymphocyte were investigated. There were less than 200 cells/µL, 200-350 cells/µL, 351-500 cells/µL and more than 500 cells/µL. The difference was considered significant with a *P-value* of less than 0.05 (95% C.I.).

RESULTS AND DISCUSSION

Absolute CD4+ T lymphocyte count between smoker and non-smoker groups

As described in Table 1, we observed that the mean value of CD4+ T lymphocyte count of the smoker group ($\bar{x}$ = 331.00 cell/µL) is slightly lower than the non-smoker group ($\bar{x}$ = 416.19 cell/µL) (*P = 0.131*). In terms of gender, we found that for male-smokers (n=18) the number of CD4+ was insignificantly lower compared with non-smokers (n=34), 331.78 cell/µL and 386.18 cell/µL, respectively. This trend was also applicable to the female group. Female-smokers (n=4) showed their mean of absolute CD4+ count as being slightly lower than that of non-smokers (n=54), 327.50 cell/µL and 435.09 cell/µL (Figure 1). It was noticeable that the mean of the CD4+ T lymphocyte count in the smoking group was less than 350 cell/µL (which is the level of absolute CD4+ T lymphocyte recommended to start anti-retroviral therapy according to the WHO), while the other group was higher. Both smoking and HIV infection are very important health issues, especially in developing countries such as Thailand. One publication showed that around 1.3% of the Thai adult population was HIV-infected. Also, publications have supported the theory that the host's immunity of HIV-infected individuals is impaired by CD4+ T cell destruction after HIV established infection. Some experiments have shown that HIV-infected smokers have a higher degree of impairment compared to those who do not smoke. Evidently, the relationship between smoking and the worsened clinical outcome of many diseases has been reported. Moreover, the quantities of CD4+ T lymphocyte were affected by smoking.

Number and percentage of population in each CD4+ T lymphocyte count level

All subjects were categorized into four groups according to their absolute number of CD4+ T lymphocyte (less than 200, 200-350, 351-500 and more than 500 cells/µL). Percentages of CD4+ in these groups were then calculated and compared. The results showed that the majority of the study population had CD4+ T lymphocyte counts in the range of 200 - 350 cell/µL – for both smoking and non-smoking groups (36.36% and 32.95%, respectively; Table 2, Figure 2). To clarify the trend of the quantities of CD4+ T lymphocyte among groups, data was rearranged into three groups: those whose number of absolute CD4+ T lymphocyte count was lower than 250 cells/µL, between 250 – 350 cells/µL and greater than 350 cell/µL. The data showed that 63.63 percent of HIV-infected smokers have their CD4+ T lymphocyte count equal to or less than 350 cells/µL (Table 3, Figure 3). While in the non-smoker group, the percentage of people who had an absolute peripheral CD4+ T lymphocyte count level higher than 350 cell/µL was equal to the lower group (Table 3, Figure 3).

According to our results, we found that cigarette smoking is insignificantly associated with the change of CD4+ T lymphocyte number in blood circulation. The mean of CD4+ T lymphocyte count in smoking HIV-infected patients was less than 350 cells/µL, while the mean of CD4+ T lymphocyte was higher than 350 cells/µL when observed in the non-smoker group. This is applicable to both genders. In the group with the level of CD4+ T lymphocyte count lower than 350 cells/µL, the percentage of population in the smoking group (63.63%) is higher than the non-smoking group (50%); on the other hand, in the group with the level of CD4+ T lymphocyte count higher than 350 cells/µL, the percentage of population in the non-smoking group is higher than the smoking group.

Table 1 - Mean of absolute CD4+ T lymphocyte between smoking and non-smoking group.

Gender	Smoking status	Number	Mean of CD4+ T lymphocyte count (cell/uL)	S.E.M.	*p- value*
male (N=52)	smoking	18	331.78	41.20	0.362
	non-smoking	34	386.18	37.00	
female (N=58)	smoking	4	327.50	170.79	0.431
	non-smoking	54	435.09	34.92	
total (N=110)	**smoking**	**22**	**331.00**	**43.38**	**0.131**
	non-smoking	**88**	**416.19**	**25.75**	

** S.E.M. is standard error of the mean; calculated by SD/sqrt(sample size)*

Figure 1 - Comparison of mean absolute CD4+ T lymphocyte count.

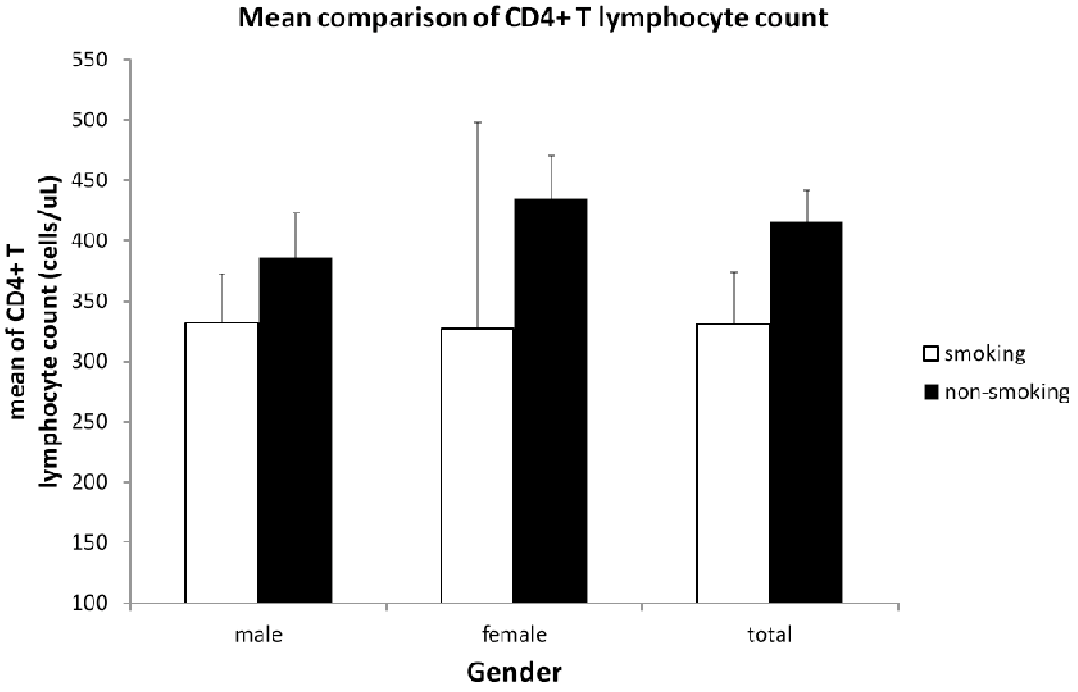
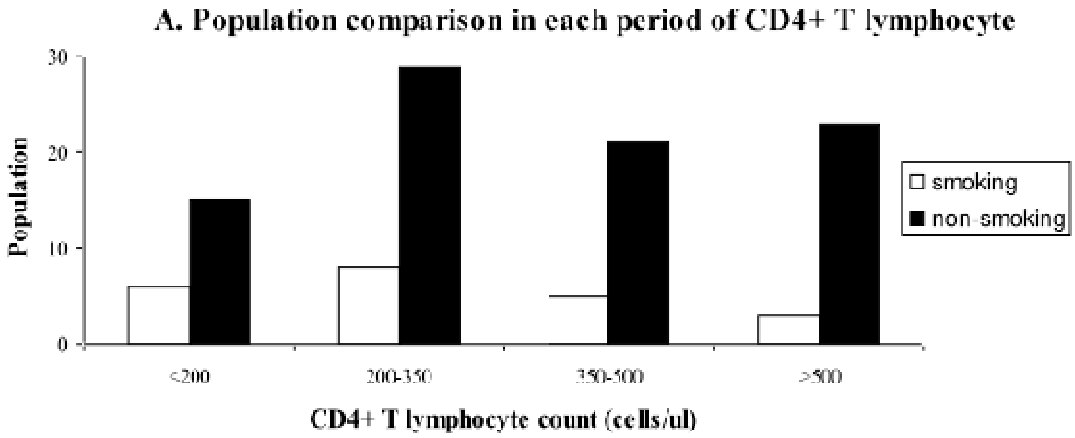


Table 2 - Number and percentage of subjects grouped by absolute CD4+ T-lymphocyte level.

CD4+ T lymphocyte count (cell/ul)	Population	
	Smoker	Non-smoker
< 200	6 (27.27%)	15(17.05%)
200-350	8 (36.36%)	29 (32.95%)
351-500	5 (22.73%)	21 (23.86%)
> 500	3 (13.64%)	23 (26.14%)
Total	**22 (100.00%)**	**88 (100.00%)**

Figure 2 - Number(A) and percentage(B) of study population in smoking and non-smoking HIV seropositive group by CD4+ T Lymphocyte level



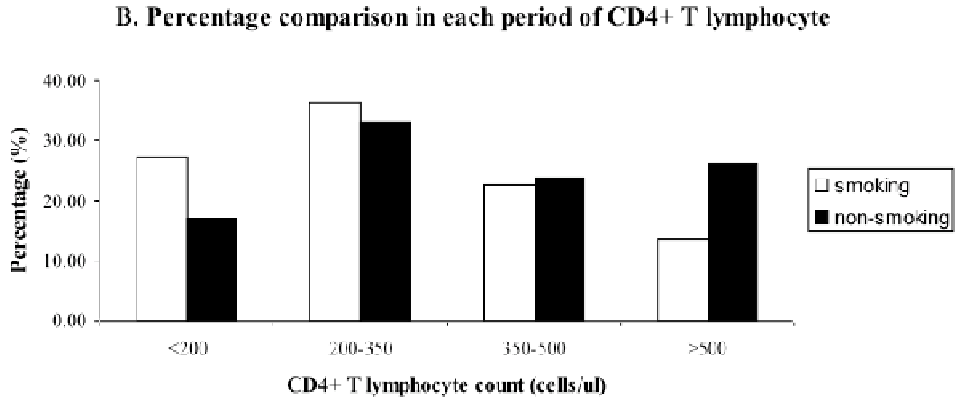
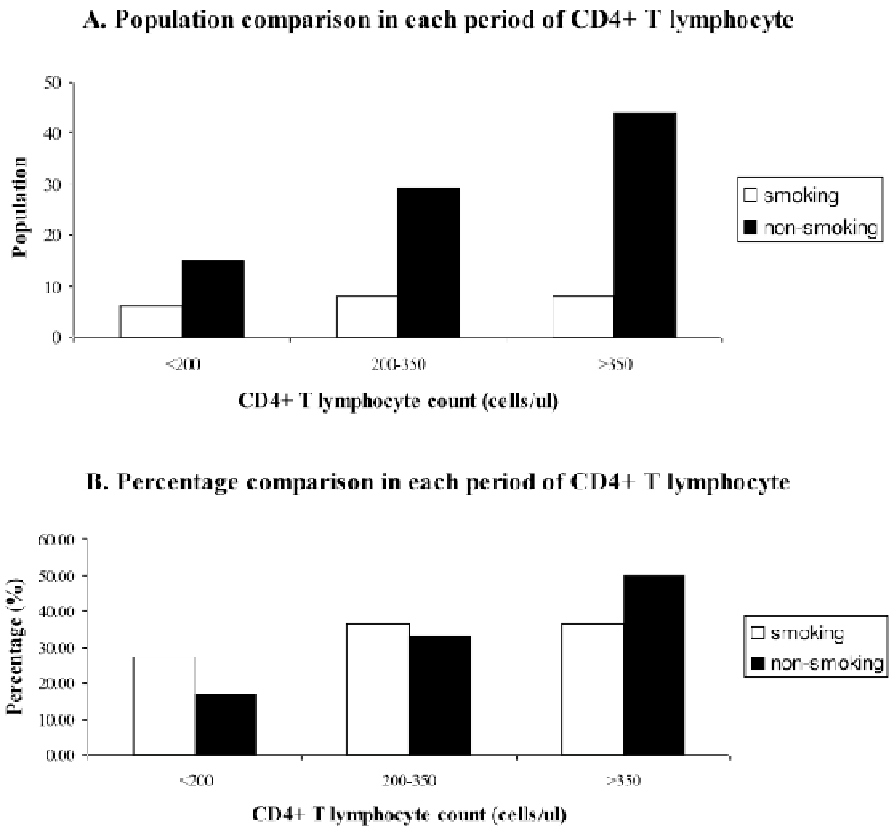


Table 3 - Number and percentage of subjects grouped by absolute CD4+ T- lymphocyte level (rearranged into three groups)

CD4+ T lymphocyte count	population			
	smoking		Non-smoking	
<200 cells/ul	6 (27.27%)	**63.63%**	15 (17.05%)	**50.00%**
200-350 cells/ul	8 (36.36%)		29 (32.95%)	
>350 cells/ul	8 (36.36%)		44 (50.00%)	
Total	22 (100.00%)		88 (100.00%)	

Figure 3 - Number(A) and percentage(B) of study population in smoking and non-smoking HIV seropositive group by CD4+ T Lymphocyte level (rearranged into three groups)



CONCLUSION

Even if there is no statistical significance in the difference of the mean CD4+ T lymphocyte count, the absolute CD4+ count at 350 cells/µL is the cut-off level for antiretroviral therapy determination according to WHO treatment guidelines. Together with the limitations of this study, including the sample size, there was a trend showing that the quality of CD4+ T cells in the smoking group is slightly lower than the non-smoking group. Further studies should be performed to gain an in-depth analysis of smoking impact on CD4+ T lymphocyte level.

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