Total Antioxidant Capacity in Plasma of HIV-Infected Patients

NANTAYA CHANARAT, M.Sc.*, PRASIT CHANARAT, M.Sc.**,
MAITREE SUTTAJIT, Ph.D.***, DUMRONG CHIEWSILP, M.D.****

Abstract

Total antioxidant capacity (TAC) of fasting EDTA plasma of 33 healthy and 64 HIV-infected patients was determined using H_2O_2-peroxidase-ABTS technique. The results revealed that the average TAC in HIV-infected patients was significantly lower than those in healthy normal persons. (0.161 ± 0.097 *vs* 0.269 ± 0.081 mmol/L Trolox equivalent, $p < 0.05$). Total lymphocytes were also counted using Hycel automatic cell counter and absolute CD4 numbers using Coulter CD4 manual kit. It was interesting that CD4 count was not correlated with the clinical symptoms of the patients. This paper suggests that prediction of severity and monitoring of the disease should be performed by determining both total lymphocyte count and total antioxidant capacity.

AIDS is a multifactorial disease and is associated with degeneration of the immune system. AIDS is caused by HIV with co-factors such as poor nutrition. It could also be called a lifestyle disease instead of an infectious disease because not all the HIV-infected patients develop AIDS until their lifestyle changes(1). Once the virus enters the body, CD4 (T helper) lymphocytes are the primary target. The virus attaches to the surface of T cell by binding to the CD4 receptor on the cell surface, infects and destroys the cells by apoptotic process. Progression of HIV infection to clinical AIDS is characterized by gradual decrease in CD4 lymphocyte count through apoptosis which in turn increases the progression to AIDS, and the risk of the opportunistic infections(2). However, there is no progression of the disease in approximately 5 per cent of the HIV-infected individuals. They still remain asymptomatic with normal or decreased CD4 counts(3). One possible hypothesis of apoptosis is that the higher the increase of oxidative stress is, the faster AIDS

* Department of Clinical Chemistry,
** Department of Clinical Microscopy, Faculty of Associated Medical Sciences,
*** Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200,
**** Department of Medical Services, Ministry of Public Health, Nonthaburi 11000, Thailand.

symptoms develop. It may be said that most of the patients die from opportunistic infections[4] or from the toxic effect of harmful drugs[1] not by HIV.

Oxidative stress occurs after an excessive production of free radicals. Free radicals are any molecules that contain one or more unpaired electrons (i.e., superoxide, hydroxyl, lipid peroxide, etc.). They are extremely reactive molecules and can cause cell injury and cell death. Free radicals can damage polyunsaturated fatty acids in the cell membrane; they also can damage proteins such as enzymes, membrane ion transporters and DNA. They are mostly produced by biochemical redox reactions involving oxygen in cell metabolism processes, by phagocytosis as a controlled inflammatory reaction and by exposure to environmental oxidants, for example UV light, pollution, cigarette smoke, alcohol drinking and many chemicals including some therapeutic drugs[5].

Free radicals initiate chain reactions which will continue until they are removed by scavengers. These are active antioxidants, both from enzyme or non-enzyme systems. The oxidative stress can be measured by determining the level of free radicals, antioxidant, or the oxidative products. This paper aims to study the TAC in plasma using the novel method modified from Miller and co- workers[6] which has not been reported elsewhere. TAC levels were compared with total lymphocytes and manual CD4 count (Coulter® Manual CD4 Count kit)[3] of HIV-infected patients for monitoring the severity of the disease.

MATERIAL AND METHOD

Venous EDTA blood was drawn from 64 HIV-infected patients, 38 males and 26 females. Plasma was separated within 1 hour and kept in -20°C until analysis of TAC. Complete blood cell count was done on Hemacel (Hycel Groupe Lisabio) Automatic Cell Counter for estimation of total lymphocytes. CD4 count was manually performed using Coulter CD4 kit. EDTA blood samples from 33 healthy persons were used as normal control group.

In principle, TAC was estimated by catalysis of standardized hydrogen peroxide with horseradish peroxidase giving active nascent oxygen atom which will oxidize [2,2' azinobis (3-ethylbenzthiazoline) sulphonate, ABTS] producing a blue color of cation ABTS radical with maximum absorption at 734 nm[8]. Standard antioxidants, Trolox (6-hydroxy-2,5,7,8-tetramethylchlorman-2-carboxylic acid) and plasma samples containing TAC caused delayed reaction. The lag time, the time before the blue color is produced, is proportional to TAC in the sample.

RESULTS

Plasma total antioxidant capacity in normal controls (mean±SD) was 0.269±0.0814 mmol/L as Trolox or 12.094±3.501 seconds. The HIV-infected patients had a significantly lower level, 0.161±0.097 mmol/L as Trolox or 7.241±4.369 seconds as lag time ($p < 0.05$) as shown in Fig. 1. It was also found that 57 per cent, 46 per cent and 29 per cent of the lag time in patients' sera which were less than 8, 6 and 4 seconds, respectively.

Total lymphocyte count in 43 patients ranged from 0.4 to 6.2 $x10^3$ cells/mm^3 and absolute CD4 count ranged from <30 to 1,292 cells/mm^3 whereas the normal values of total lymphocyte count and CD4 count ranged from 1.2 to 3.4 x 10^3 and 700 to 1,200 cells/mm^3, respectively.

The concurrent of total antioxidant capacity as lag time and total lymphocyte or CD4 count were studied. Total lymphocyte count below normal with low TAC was found in 30 per cent and low CD4 count with low TAC in 26 per cent of the patients as shown in Table 1.

DISCUSSION

It has been demonstrated that there is a significant increase in the percentage of apoptosis cells among the circulating CD4 lymphocytes in HIV-infected patients and the frequency of apoptosis increases with the progression of infection[9].

The true cause of AIDS is still controversial. There are many cases of HIV-free acquired immunodeficiency reported and evidence has been accumulated suggesting that HIV-infected patients are under chronic oxidative stress[10]. The antioxidative defense systems decline including changes in levels of vitamin C, vitamin E, carotenoids, selenium, superoxide dismutase and glutathione. Elevation of hydroperoxides and malondialdehyde (MDA) are the indicative markers of the process. Many researchers are intensively investigating to solve this problem. There are many trials with supplementation of antioxidants from herbs and natural food products[5] including curcumin, ß-carotene, N-acetylcysteine, glutathione, scavenger-

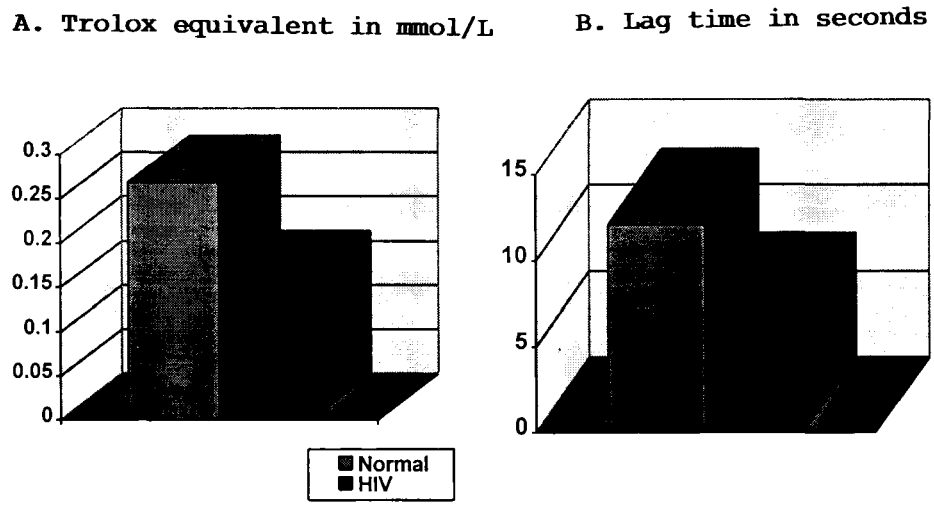

Fig. 1. Mean total antioxidant capacity in plasma of normal individuals (n=33) and HIV-infected patients (n=43). A. as Trolox equivalent in mmol/L and B. as lag time in seconds.

Table 1. Percentage of HIV-infected patients with various total lymphocyte (n=43), CD4 count (n=30) and TAC as lag time in seconds.

Parameter	Percentage of HIV - infected patients	
	TAC < 8 sec	TAC ≥ 8 sec
Total lymphocyte count (cell/mm^3)		
< 2,000	30.2	30.2
> 2,000	18.6	27.9
CD4 (cell/mm^3)		
< 200	26.6	30.0
> 500	6.6	10.0

vitamin, coenzyme Q, bioflavonoids as a method to enhance immune function and halt HIV disease progression(11,12).

The reaction used in TAC determination was modified by using horseradish peroxidase instead of methmyoglobin for catalysis of hydrogen peroxide that will change ABTS coloration. Peroxidase reacts with hydrogen peroxide and gives many colored complexes as described formerly(13) therefore the final absorbance does not correlate with TAC. We measured the lag time before the color reaction occurred, and found that it corresponded very well with TAC levels. The HIV-infected patients had significantly shorter lag time than that of normal controls(8).

This study confirmed our previous report in which plasma of 60 HIV-infected patients was determined for vitamin E, vitamin A, ß carotene and MDA compared to 32 healthy normal persons. Antioxidant vitamins, vitamin E and ß carotene, significantly decreased in HIV-positive patients while lipid oxidative product, MDA increased(5).

The combination of two parameters, such as total lymphocytes and TAC, CD4 and TAC, can be used as a prediction of the clinical outcome. Other markers of oxidative stress, for example superoxide radical, thiol substance, reduced glutathione and superoxide dismutase could be also used as a predictor as well.

This preliminary study gave us knowledge of antioxidant supplementation to reduce oxidative stress in the patients. The supplementation of antioxidants will be an alternative way to slow down the progression of HIV infection to AIDS. In the future, the HIV-infected patients can have a longer span of symptom-free life and can have a better quality of life as long as they maintain a healthy lifestyle.

(Received for publication on July 11, 1997)

REFERENCES

1. Phoenix AZ. What if everything we have been told about HIV and AIDS is a lie? ECHO Magazine, Oct 31, 1996.
2. Landvik S (correspondence). Potential beneficial role of antioxidants in HIV Infection and AIDS. Veris Research Summary. May 1995.
3. Duesberg PH. HIV is not the cause of AIDS. Science 1988; 241: 514-7.
4. Willner RE. Deadly deception, the proof that sex and HIV absolutely do not cause AIDS. Boca Raton, FL, Peltec Publ Co, 1994.
5. Suttajit M, Chanarat N, Chanarat P. Biochemical approach for AIDS treatment by natural antioxidants in diet. In: Proceedings of the 7th Asian-Pacific Congress of Clinical Biochemistry. 1995; 323-7.
6. Miller NJ, Rice-Eran C, Davies MJ, Gopihathan V, Milner A. A novel method for measuring antioxidant status in premature neonates. Clin Sci 1993; 84: 407-17.
7. Coulter® Manual CD4 Count kit, Coulter Corporation, 1993.
8. Tungsen, N. Total antioxidant capacity in serum of HIV-infected patients. Term Paper for B.Sc. (Medical Technology), Faculty of Associated Medical Science, Chiang Mai University, 1996; 29-30.
9. Wood KA, Youlse RJ. Apoptosis and free radicals. In : The Neurobiology of NO° and °OH, Chineh CC, Gilbert DL, Cotton CA, (eds). Annals NY Acad Sci 1994; 738: 400-7.
10. Pace GW, Leaf CD. The role of antioxidative stress in HIV disease. Free Radic Biol Med 1995; 19: 523-8.
11. Delmas-Beauvieux MC, Peuchant E, Couchouron A, et al. The enzymatic antioxidant system in blood and glutathione status in HIV-infected patients : effects of supplementation with selenium and beta-carotene. Am J Clin Nutr 1996; 64: 101-7.
12. Roberts RL, Aroda VR, Ank BJ. N-acetylcysteine enhances antibody-dependent cellular cytotoxicity in neutrophils and mononuclear cells from healthy adults and immunodeficiency virus-infected patient. J Infect Dis 1995; 172: 1493-502.
13. George P, Irvine DH. The reaction between methmyoglobin and hydrogen peroxide. Biochem 1952; 52: 511-7.

ความสามารถในการเป็นแอนติออกซิแดนท์โดยรวมในพลาสมาของผู้ป่วยติดเชื้อเอชไอวี

นันทยา ชนะรัตน์, วท.ม., ประสิทธิ์ ชนะรัตน์, วท.ม.**,*
*ไมตรี สุทธจิตต์, Ph.D.***, ดำรง เชี่ยวศิลป์, พ.บ.*****

ได้ศึกษาความสามารถในการเป็นแอนติออกซิแดนท์โดยรวม (Total antioxidant capacity, TAC) ในพลาสมาที่มี EDTA เป็นสารกันเลือดแข็ง จากคนปกติ 33 คน และจากผู้ป่วยติดเชื้อเอชไอวี จำนวน 64 ราย โดยวิธี H_2O_2-peroxidase-ABTS technique พบว่าค่าเฉลี่ยของ TAC ในผู้ป่วยติดเชื้อเอชไอวี มีระดับแอนติออกซิแดนท์โดยรวมต่ำกว่ากลุ่มคนปกติอย่างชัดเจน (0.161±0.097 *vs* 0.269±0.081 mmol/L Trolox equivalent, $p<0.05$) เมื่อนับจำนวนลิมโฟซัยท์ทั้งหมดโดยใช้เครื่อง Hycel Automatic Cell Counter และนับจำนวน CD4 ทั้งหมดโดยใช้ Coulter CD4 manual kit พบข้อที่น่าสนใจคือ จำนวน CD4 ทั้งหมดไม่สอดคล้องกับอาการทางคลินิกของผู้ป่วย รายงานนี้เสนอแนะว่าการตรวจนับจำนวนลิมโฟซัยท์ทั้งหมดร่วมกับระดับความสามารถในการเป็นแอนติออกซิแดนท์โดยรวม อาจช่วยในการทำนายความรุนแรงของอาการในผู้ป่วยคิดเชื้อเอชไอวีได้

* ภาควิชาเคมีคลินิก,
** ภาควิชาจุลทรรศนศาสตร์คลินิก, คณะเทคนิคการแพทย์,
*** ภาควิชาชีวเคมี, คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, เชียงใหม่ 50200
**** กรมการแพทย์, กระทรวงสาธารณสุข, นนทบุรี 11000