BIOCHEMICAL AND HEMATOLOGICAL MANIFESTATIONS OF HIV/AIDS IN CHIANG MAI, THAILAND

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Abstract. HIV/AIDS is a multifactorial and multi-step disease. No single treatment against AIDS can save a patient. Our last report showed that vitamin A, vitamin E and β-carotene were decreased while malondialdehyde (MDA) was increased. This report aims to evaluate biochemical and hematological parameters in HIV/AIDS patients in Chiang Mai, Thailand by holistic approaches. Sera from HIV/AIDS patients were examined for sugar, cholesterol, uric acid, total protein, albumin, urea, creatinine, AST, ALT, ALP, total/direct bilirubin, vitamin E, MDA, total antioxidant capacity (TAC), β-carotene, complete blood cell counts, platelet count, CD4 count, prothrombin time, partial prothrombin time and soluble *Fas* (s*Fas*). The results found that s*Fas* levels in sera prior to holistic approach was not different from reference values and not significantly correlate with CD4 and absolute lymphocyte count. s*Fas* could not serve as putative marker for CD4 destruction. After 3 months CD4 count, MDA, vitamin E and TAC did not change statistically. This approach had no effect on liver and kidney functions, red blood cell, white blood cell, platelet counts, and blood clotting factors. This presentation may be some alternative approaches to combat HIV infections and AIDS, leading to stabilize or extend survival time which should further be elucidated.

INTRODUCTION

AIDS epidemic in the early 1980s, some 50 million individuals worldwide have been infected with HIV, of whom one-third have already died. In 1999, 2.6 million of death due to AIDS, 5.6 million of new HIV infection and 33.6 million of people living with HIV/AIDS are the global impact of HIV/AIDS around the world (Digby, 2000).

In Thailand, the number of new AIDS cases reported to the Ministry of Health was 25,000 in 1997 and 18,000 in 1998, a third of these cases are in the northern part. The main cause of CD4+ T-lymphocyte depletion in AIDS is apoptosis. Various agents appear to be able to trigger apoptosis of CD4+ T cells, including viral protein (*ie* gp120, Tat, etc), inflammatory cytokines (*ie* TNF-α), toxin produced by opportunistic microorganisms, and oxidation stress. AIDS patients present low levels of antioxidants, *ie*, vitamin E, vitamin C, selenium and glutathione, etc, most likely due to poor diet in antioxidants, alcohol, smoking, drug consumption, digestive problems, and the coadministration of the antiviral drug zidovudine, while lipid peroxidation products, *ie*, malondialdehyde (MDA), was increased (Patrick, 1999; Suttajit *et al*, 1995). Increased lipid peroxidation induced by reactive oxygen species may play a role in the stimulation of HIV replication. Therefore, the aim of this study considered about antioxidant, anti-apoptosis, along side natural antiviral strategies in order to combat AIDS.

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MATERIALS AND METHODS

HIV-infected patients in Chiang Mai, Thailand were directed to drink a mixture "MG" herb's tea, supplement with vitamin E (200 IU/day) and N-acetylcysteine (NAC 1,000 mg/day) every day AIDS patients were told to live in clean environment, slightly exercise, no smoke, no alcohol and keeping non-stressed

emotion with enough sleeping, etc, for improvement of their lifestyle. Blood were drawn before and after intervention for 3 months to determine changes of hematological and biochemical parameters.

Hematological parameters were complete blood cell counts (CBC), platelet count, prothrombin time (PT), partial thromboplastin time (PTT). All were done using standard methods. CD4 count were performed in EDTA blood under the manufacturer's instruction of COULTER Manual CD4 kit, Florida, USA.

Biochemical parameters: Sugar, cholesterol, uric acid, total protein, albumin, urea, creatinine, AST, ALT, ALP, total/direct bilirubin were done on Abbott Spectrum CCx analyzer (Abbott Spectrum CCx, 1968). Serum vitamin E and β-carotene were determined by modified Bieri's method (Chanarat *et al*, 1981). MDA in sera were estimated using thiobarbituric acid reaction (Smith *et al*, 1993). Total antioxidant capacity (TAC) in serum were done using the principle that antioxidant can delay the oxidation of 2,2′-azino bis-(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) by hydrogen peroxide. The amount of antioxidant were estimated by comparison of lag time (time before the reaction starts) with the 6-hydroxy-2,5,7,8-tetramethylchlorman-2-carboxylic acid (Trolox) standard (Nicholas *et al*, 1993). The soluble *Fas* (s*Fas*) in the sera were measured with a sandwich ELISA kit (Medical and Biological Laboratories Co., Nagoya, Japan) according to the manufacturer's instructions (Seishima *et al*, 1996).

RESULTS

After 3 months there were 87 patients studied for comparison of biochemical changes. It was found that there were no effect of herbs on all parameters studied, except only β-carotene increased as shown in Table 1. TAC was not correlate with absolute lymphocyte and CD4 numbers.

For hematological parameters it was found that RBC, WBC or platelet did not decrease while CD4 was not increased but PT and PTT

Table 1
Biochemical manifestation after holistic approaches for 3 months.

Parameters		Before			After		
		No.	$\bar{X}$	SD	No.	$\bar{X}$	SD
Glucose	mg/dl	89	79.28	13.25	87	78.78	11.69
BUN	mg/dl	89	9.38	3.13	87	10.71	4.14
Creatinine	mg/dl	89	0.89	0.20	87	0.91	0.19
Cholesterol	mg/dl	89	155.5	33.91	87	151.30	29.36
Uric acid	mg/dl	89	5.70	4.10	87	5.63	1.26
Total protein	g/dl	89	8.06	0.96	87	8.49	0.92
Albumin	g/dl	89	3.95	0.59	87	4.14	0.66
ALT	U/l	89	28.56	23.66	87	15.15	15.11
AST	U/l	89	35.88	20.72	87	38.30	17.19
ALP	U/l	89	70.21	34.59	87	70.41	38.49
Total bilirubin	mg/dl	89	0.63	0.26	87	0.61	0.22
D. bilirubin	mg/dl	89	0.57	0.18	87	0.54	0.16
MDA	mM	89	28.09	7.48	87	29.14	16.84
Vitamin E	mg/dl	77	1.25	0.62	49	1.80	1.20
β-carotene	μg/dl	76	332.3	168.68	49	408.26	298.36
TAC	second	89	8.91	6.61	50	9.38	8.02
TAC	mM	89	0.27	0.20	50	0.29	0.24

Table 2
Hematologic manifestation after holistic approaches for 3 months.

Parameters	Before			After		
	No.	$\bar{X}$	SD	No.	$\bar{X}$	SD
WBC x 10^3/µl	296	5.63	2.05	129	5.50	2.01
Absolute Lymphcyte x 10^3/µl	296	1.66	0.84	129	1.70	0.76
RBC x 10^6/µl	296	4.30	0.75	129	4.38	0.69
Hb g/dl	296	11.24	2.10	129	11.53	1.83
Hct %	296	34.57	6.00	129	35.58	5.28
Plt x 10^3/µl	296	248	90	129	243	78
CD4 cells/µl	75	204	281	41	269	389
PT second	64	16.74	2.65	36	17.53	2.63
PTT second	27	45.20	17.75	22	45.53	8.65

Table 3
Percent of HIV/AIDS patients (n = 60) at different levels of s*Fas*.

Cells/µl	No.	s*Fas* (ng/ml)			$\bar{X}$ ± SD
		(<1)	(1-1.5)	(>1.5)	
Absolute Lymphocyte					
<1,000	11	7	25	5	1.19 ± 0.28
1,000-2,000	29	85	66	78	1.07 ± 0.37
>2,000	20	7	7	15	1.17 ± 0.28
CD4					
<200	29	20	8	13	1.15 ± 0.29
200-400	12	73	75	73	1.15 ± 0.33
>400	19	6	16	13	1.17 ± 0.28

were slightly increased (Table 2).

s*Fas* levels (Mean±SD) of the patients was not statistically different from reference value (1.22±0.58 *vs* 0.93±0.5 ng/ml) and not correlated to CD4 and absolute lymphocyte numbers as shown in Table 3.

DISCUSSION

It is believed that CD4 cell death occurs from apoptosis. In fact it may be multiple pathways leading to cell death in T cells from HIV-1 infected individuals. Recently, the *Fas* receptor (*Fas*, APO-1/CD95) and its ligand (*Fas*L, CD95L), which are transmembrane protein of TNF family of receptors and ligands, play a role in engagement of *Fas* by *Fas*L triggers a cascade of subcellular events that significance in program cell death (apoptosis). Our results showed that s*Fas* was not correlate with CD4 cell destruction. We hypothesized that reactive oxygen species (ROS: H_2O_2, O_2^-, OH^-) may be one of major roles on CD4 apoptosis and destruction which may be reduced by natural antioxidants from herbs in our patients.

Befil *et al* (1999) described a novel mechanism of activation induced cell death, that has many events of necrosis, as activation-associated necrosis (AAN). High level of AAN could predict AIDS and long term risk of death independently of the CD4 count.

Some cases of our series, CD4 count were still below 200 cells/µl for longer than 2 years.

It is possible that HIV-1 interferes with nucleotide synthesis. It seems likely that one or several viral proteins might be involved rather than whole virus itself leads to poor proliferation capacity of CD4 cells.

HIV-1 not only causes low CD4 count but it also associates with granulocytopenia, anemia, loss of specific cytotoxic T lymphocyte (CTL) and antibody specific response, impaired cell division of keratinocyte, gut epithelium, hair follicles, and mucosal cell. A future strategy should find out some substances for prevention of aberrant metabolism in proliferating cells as a consequence of HIV-1 infection. A holistic approach by pluristic medicine may be suitable for our patients or those in other developing countries.

REFERENCES

Abbott Spectrum CCx. Reagents, Abbott Laboratories, Diagnostics Division, 1968.

Befil M, Borthwick NJ, Simmonds HA. Novel mechanism for the impairment of cell proliferation in HIV-1 infection. *Immunol Today* 1999; 20: 258-61.

Chanarat N, Boonmee Y, Jitrthai S, Haesungcharern A. Vitamin E. I. Simple method for determination of vitamin E in serum. *Bull Chiang Mai AMS* 1981; 14: 14.

Digby PW. The statistics of HIV/AIDS: Seeking the facts. *Chiang Mai Newsl* 2000; 9: 23.

Nicholas JM, Catherine RE, Michael JD, *et al*. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* 1993; 84: 403-12.

Patrick L. Nutrients and HIV : part one - beta carotene and selenium. *Altern Med Rev* 1999; 4: 6, 403-13.

Seishima M, Takemura M, Saito K, *et al*. Highly sensitive ELISA for soluble *Fas* in serum : Increase soluble *Fas* in the elderly. *Clin Chem* 1996; 42: 1191-4.

Smith CV, Hansen TN, Martin NE, McMicken HW, Elliot SJ. Oxidant stress responses in premature infants during exposure to hyperoxia. *Pediatr Res* 1993; 34: 360-5.

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.