CHAPTER 2

LITERATURE REVIEWS

Human Immunodeficiency Virus (HIV) is the pathogen which causes Acquired Immunodeficiency Syndrome (AIDS). The period of time taken for people infected with HIV to develop AIDS is varied so that there are many factors involved in the pathogenesis of HIV infection, including the basic role of host nutrition. Therefore, the current reviews are to present the relation between role of micronutrients in HIV pathogenesis and immune system's response pathogenesis of HIV infection, highly active antiretroviral therapy (HAART) for HIV/AIDS patients.

2.1 Immune system and HIV infection

2.1.1 Immune system's response and pathology of HIV infection

HIV virus is transmitted through direct exchange of body fluids then macrophages and dendritic cells on the surface of mucous membranes bind virus and shuttle it into the lymph nodes, which contain high concentrations of CD4+ T cells and regulates immune response [23]. CD4+ T cells are the main of HIV infected cell. These cells play a crucial role in the immune system, by coordinating the actions of

other immune system cells. Over time, HIV infection leads to a severe reduction in the number of T helper cells which available to help fight disease. Factors of immune deficit in HIV infection are as follow; increasing HIV replication and CD4+ T cells depletion [24, 25].

CD4+ T cells are the ordination of the immune system. They produce cytokines and help the effectors of the innate immunity, such as natural killer cells (NK), monocytes in the elimination of virus-infected cells. In addition, they are essential to the specific activation and maturation of B-lymphocytes into antibody-secreting plasmocytes. They are required for the differentiation of CD8+ T cells into virus-specific cytotoxic T-lymphocytes (CTL). Finally, they are the source of chemokines, which are suppressor factors of HIV replication. CD4+ T cells destruction can be mediated directly by immune system such as cell apoptosis and imbalance activation of CD4+ T cells (Figure 2.1). Therefore, the progressive disappearance of CD4+ T cell during infection with HIV leads to the lack of control of HIV replication and to the development of the severe immune deficiency responsible for the occurrence of opportunistic infections associated with AIDS [24, 25].

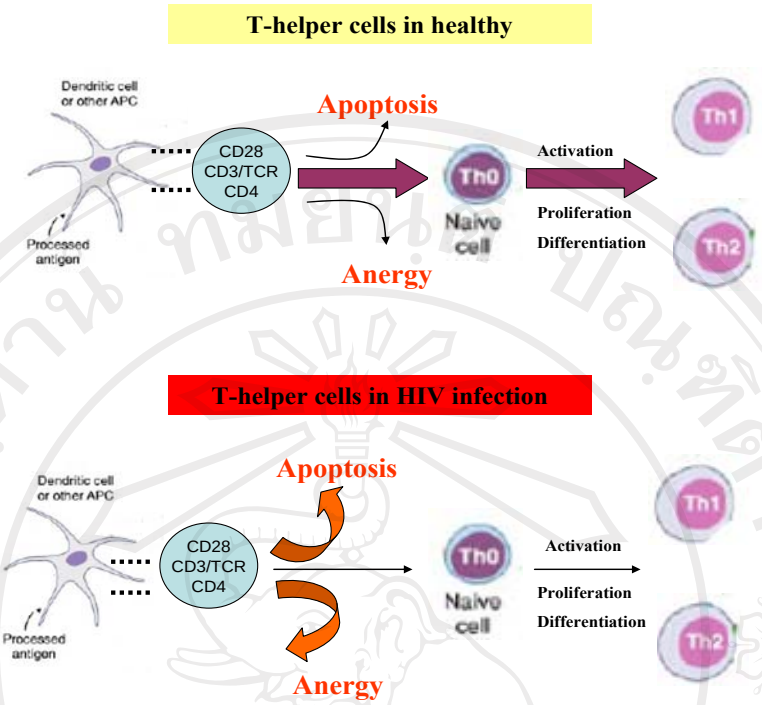


Figure 2.1 Comparison of activation, proliferation and differentiation of T-helper cells in healthy and T-helper cells in HIV infection.

(Source: จันทพงษ์ วะสี. คณะแพทยศาสตร์ศิริราชพยาบาล. 2541)

T-helper cell (Th) in healthy, signal from APC to Th, can stimulate the balance Th1 and Th2 immune response while Th in HIV infection is unsuitable to stimulate. Therefore, most Th cell is destroyed by apoptosis or anergy.

2.1.2 AIDS Definition

In 1993, the CDC revised classification system for HIV-infected adolescents and adults with a CD4+ T cell counts. Definition of AIDS includes all HIV-positive people with CD4+ T cell counts below 200 cells/μL of blood. The system is based on ranges of CD4+ T cell counts which are separated three categories as show in Table 2.1.

Table 2.1 AIDS surveillance case definition for adolescents and adults: 1993 [26]

CD4+ T cell categories	Clinical Categories		
	A	B	C*
	Asymptomatic, or PGL, or acute HIV infection	Symptomatic ϕ (not A or C)	AIDS indicator condition
500 /mm^3 (≥ 29%)	A1	B1	C1
200 to 499/mm^3 (14% to 28%)	A2	B2	C2
< 200/mm^3 (<14%)	A3	B3	C3

* All patients in categories A3, B3, and C1-3 are defined as having AIDS based on the presence of an AIDS-indicator condition, Table 2.2 and/ or a CD4 Cell count < 200/mm^3.

ϕ Symptomatic conditions not included in category C that are a) attributed in HIV infection or indicative of a defect in cell-mediated immunity or b) considered to have a clinical course or management that is complicated by HIV infection. Example of B conditions include, but are not limited to, bacillary angiomatosis; thrush; vulvovaginal candidiasis that is persistent, frequent or poorly responsive to therapy; cervical dysplasia (moderate or severe); cervical carcinoma in situ; constitutional symptoms such as fever (38.5 °C) or diarrhea > 1 month; oral hairy leukoplakia; herpes zoster involving two episode or >1 dermatome; idiopathic thrombocytopenic purpura (ITP); listeriosis; pelvic inflammatory disease (PID) (especially if complicated by a tubo-ovarian abscess); and peripheral neuropathy.

2.2 When to start highly active antiretroviral therapy (HAART) in HIV-infected patients

2.2.1 Guidelines to initiate HAART in Thailand

Highly active antiretroviral therapy (HAART) is combination therapy against HIV infection, which typically includes three drugs from at least two different classes. There are three main classes of drugs: Protease inhibitors (PIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs) and Nucleoside reverse transcriptase inhibitors (NRTIs). The benefits of HAART have been linked with decreasing rates of AIDS opportunistic infections, improving quality of life and increasing survival [(27)]. Thailand is one of the countries that use HAART for treatment AIDS patients. Thai Government Pharmaceutical Organization (GPO) has introduced an antiretroviral drugs regimen, GPO-vir. The GPO-vir which is a combination of triple drugs contains stavudine (d4T), lamivudine (3TC) and nevirapine into a single pill. Regimens for initial therapy are summarized according to guidelines of Thailand, 2004. Recommendations are based on CD4+ T cell count and symptoms, as indicated in Table 2.2 [(28)].

Table 2.2 Guidelines to initiate GPO-vir (Thailand-2004) [28]

Clinical Category	CD4 cell count	Plasma HIV RNA	Recommendation
Symptomatic (AIDS or severe symptoms)*	Any value	No screening test	Treat
No symptom of AIDS**	< 250/mm^3	No screening test	Treat
Asymptomatic	< 200/mm^3	No screening test	Treat
Asymptomatic	> 200/mm^3	No screening test	No treat

Remarks; * AIDS defining symptom (see Appendix A).
** Oral thrush, unexplained fever, unexplained diarrhea > 14 days, rush, Weight loss >15% in 3 months.

2.2.2 Other guidelines to initiate HAART

There are a number of guidelines for indication to initiate antiretroviral therapy including Department of Health and Human Services (DHHS) Guidelines, The International AIDS Society-United States of America (IAS-USA) Guidelines and World Health Organization (WHO) Guidelines [26] as shown in Table 2.3, 2.4 and 2.5.

Table 2.3 Indications to initiate antiretroviral therapy-DHHS guidelines

Clinical Category	CD4+ T cell count	Plasma HIV RNA	Recommendation
Symptomatic (AIDS or severe symptoms)*	Any value	Any value	Treat
Asymptomatic	< 200/mm^3	Any value	Treat
Asymptomatic	< 200 to 350 /mm^3	Any value	Treatment should be offered; controversy exists for patients with viral load < 20,000 c/mL due to low probability of AIDS-definiing diagnosis within 3 years.
Asymptomatic	> 350/mm^3	≥ 100,000 copies/mL	Most clinicians would defer therapy

* Unexplained fever or diarrhea > 2 - 4 weeks, thrush or unexplained weight loss of 10% baseline weight.

Table 2.4 Indications to initiate antiretroviral therapy: IAS-USA guidelines - 2004

Disease Stage	Recommendations
Symptomatic HIV	ART recommended
Asymptomatic ▪ CD4+ T cell counts < 200/mm^3	ART recommended
▪ CD4+ T cell counts 200- 350/mm^3	ART considered. Defer if viral load (VL)low, CD4+ T cell counts slop < 50 /mm^3/year, patient reluctance. Treat if VL > 100 /mm^3/year
CD4+ T cell counts > 350/mm^3	Usually defer. Consider if high viral load or rapid CD4+ T cell counts slope.

Table 2.5 Indications to initiate antiretroviral therapy: WHO guidelines

CD4+ T cell counts count available
WHO stage IV* (AIDS - defining diagnosis)
WHO stage III (including HIV wasting, chronic enigmatic diarrhea, chronic enigmatic FUO, active pulmonary tuberculosis, recurrent invasive bacterial infections or recurrent/persistent mucosal candidiasis) with consideration of using CD4+ T cell counts < 350/mm^3 to assist decision making.
WHO stage I-II* plus CD4+ T cell counts <200/mm^3
CD4+ T cell counts not available
WHO stage IV*
WHO stage III (including HIV wasting, chronic enigmatic diarrhea, chronic enigmatic FUO, active pulmonary tuberculosis, recurrent invasive bacterial infections or recurrent/persistent mucosal candidiasis regardless of TLC)
WHO stage II * plus TLC < 1200 cells/mm^3

* Clinical stage

- Clinical stage I: Asymptomatic or PGL, and/or normal activity
- Clinical stage II: Weight loss < 10%, minor mucocutaneous conditions, zoster < 5 years, recurrent URIs, and/or symptomatic plus normal activity.
- Clinical stage III: Weight loss >10%, unexplained diarrhea > 1 month, unexplained fever > 1 month, thrush, oral hairy leukoplakia, pulmonary TB in past year, or severe bacterial infection, and/or bedridden < 50% of days in the past month.
- Clinical stage IV: CDC-defined AIDS and/or bedridden > 50% of days in the past month.

2.3 Micronutrients and the pathogenesis of HIV infection

Micronutrients play a critical role in the maintenance of immune function and overall metabolism. They are susceptible to depletion during infections, including HIV infection. The role of micronutrients deficiency in pathogenesis of HIV infection is base on the free radical theory and the nutritional immunological theory. Two theories have related intracellular oxidative stress, enhanced viral replication and a reduction in the number of circulating CD4+ T cell count which associate with individual or accumulated nutrient deficiency [29].

2.3.1 Free radical theory and HIV pathogenesis

Free radicals are generated in the body by cellular respiration, environment exposures, immune effectors cell or injured tissue. However, free radicals are balanced by the antioxidant defense system (Figure 2.2). Oxidative stress refers to the condition when the balance between free radicals and antioxidants is disorder. They cause to damage bystander cells and induce pathology [30].

There are two causes of increasing free radicals generation in HIV infection. Firstly, the virus entry to the cell which disturbs the normal physiological biochemistry of the endoplasmic reticulum and mitochondria with increased free radical generation. Secondly, during phagocytosis of microorganism by macrophages and neutrophils activate to kill the virus through the generation of oxygen free radicals such as super oxide radicals ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). [31, 32]

Oxidative stress responses to the presence of cytokines and hydroxyl radicals which can trigger the activation of nuclear factor kappaB (NF-kB) [33-35]. NF-kB is a transcriptional promoter in lymphocytes and macrophage. It activates genes in the nuclear of these cells which can induce HIV replication, damage DNA and lead to apoptosis. NF-kB also binds to HIV provirus gene material in the nuclease of HIV infected cells and activates HIV replication. Viral replication, in turn, increases cellular levels of cytokines, like tumor necrosis factor-alpha (TNF-∝), that promote the production of free radicals and the activation of NF-kB, initiating a vicious cycle of viral replication and free radical production [34]. The influencing of oxidative stress and immune deficiency in HIV infection is shown in Figure 2.3.

2.3.2 Nutrition immunological theory and HIV pathogenesis

Some micronutrients play essential roles in maintaining normal immune function. According to this theory, micronutrient deficiencies compromise host immunity to HIV and associate infections which lead to clinical progression of disease. In patients with HAART, viral suppression is not always accompanied by complete immune reconstitution. Micronutrient deficiencies may contribute to the pathogenesis of HIV infection through increased oxidative stress and compromised immunity. Several studies reported that low levels of plasma or serum vitamin A, E, B12, carotenoid, zinc and selenium have been associated with decreased CD4+ T cell count and increased mortality rate [48, 54]. This review will report on the impact of micronutrients in HIV-infected patients on HAART.

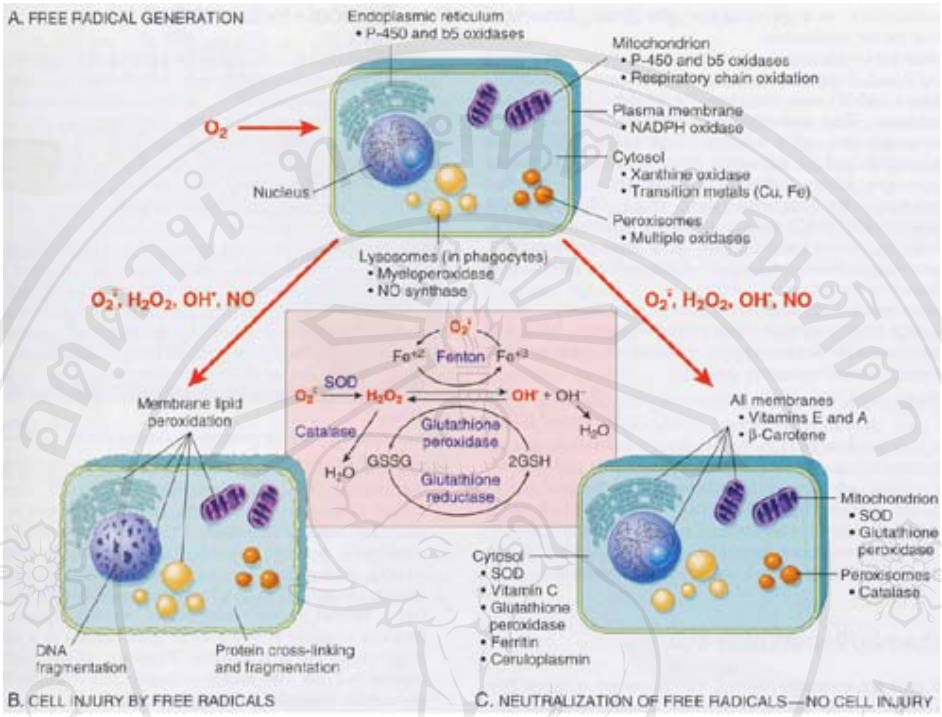


Figure 2.2 The generation of free radicals and antioxidant defense system in human body

(Source: Basic pathology. 7th edition, Elsevier Science, 2003)

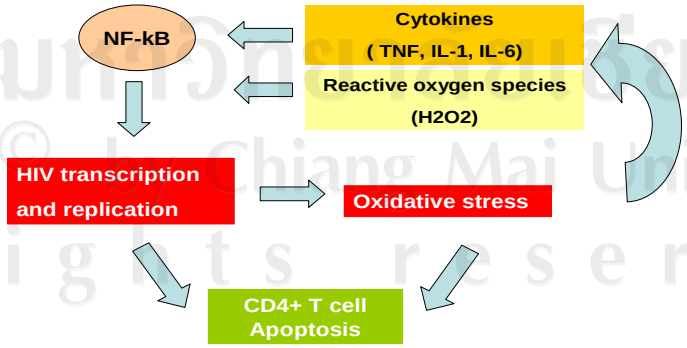


Figure 2.3 A vicious cycle of HIV infection and oxidative stress

2.4 The role of micronutrients in human

The roles of micronutrients have been associated with several systems in human body. Our study is focus on effects of vitamin A, E, B12, zinc and selenium on immune function.

2.4.1 Vitamin A

2.4.1.1 General information of vitamin A

Vitamin A refers to a group of fat-soluble substances. The biological activity of the parent substance of the group called all- *trans* retinol or retinol. Certain carotenoids, such as beta-carotene, alpha-carotene and beta-cryptoxanthin are dietary precursors of vitamin A. These substances are called pro-vitamin A and the structure of all-trans retinol is shown in Figure 2.4.

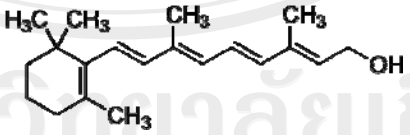


Figure 2.4 Structure of all- *trans* retinol

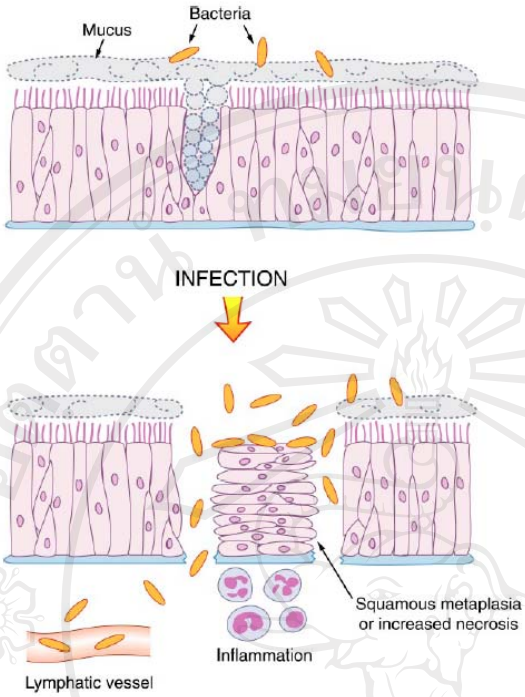
Vitamin A is presented in many animal tissues and is readily absorbed from dietary sources in the terminal small intestine. Liver is the richest dietary sources of vitamin A. Plants do not contain vitamin A, but many dark-green or dark-yellow plants contain carotenoids such as beta-carotene that serve as provitamins. Since, they

are converted within the intestinal mucosa to retinol during absorption. Vitamin A is stored in the liver as retinyl esters and, when needed, exported into blood, where it is carried by retinol binding protein for delivery to other tissues [36]. The recommended daily allowance (RDA) for vitamin A is 5,000 international units (IU) for adults [37].

2.4.1.2 Role of vitamin A on immune function in HIV infection

Vitamin A or retinol and its metabolites are commonly known as the anti-infective vitamin because they are required for normal function of the immune system. Retinol and retinoic acid (RA) are important substance in cell differentiation by activation of DNA receptors which lead to gene expression for variety of structural proteins. Therefore, vitamin A is necessary for the production, structure and normal function of cells, including T and B cell antibody response and maintenance of mucosal epithelia cell [38-40].

Vitamin A deficiency can impair regeneration of normal mucosal epithelial barriers during infection by influence to monocyte differentiation and reduce the numbers of NK cells and impairs their cytolytic activity (Figure 2.5) [38]. In addition, vitamin A deficiency leads to change the pattern of Th1/Th2 cytokine production. Retinoic acid deficiency decreases the production of the Th1-enhancing cytokines Interleukin 12 (IL-12) and interferon-gamma (IFN-γ). Thus, it enhances indirectly Th2 development because these cytokines down-regulate Th2 response and also impairs antibody response to antigens, as shown in Figure 2.6 [38].



Normal mucosal epithelium (top) prevents adherence of many potentially pathogenic bacteria by trapping them in mucus and moving them out of the airways or down the gastrointestinal tract.

In vitamin A deficiency, mucosal epithelium that is damaged by infection (and the associated inflammation) does not regenerate normally

Figure 2.5 Vitamin A and mucosa immunity

(Source: Vitamin A, infection, and immune function. Annu. Rev. Nutr.2001; 21: 167-192)

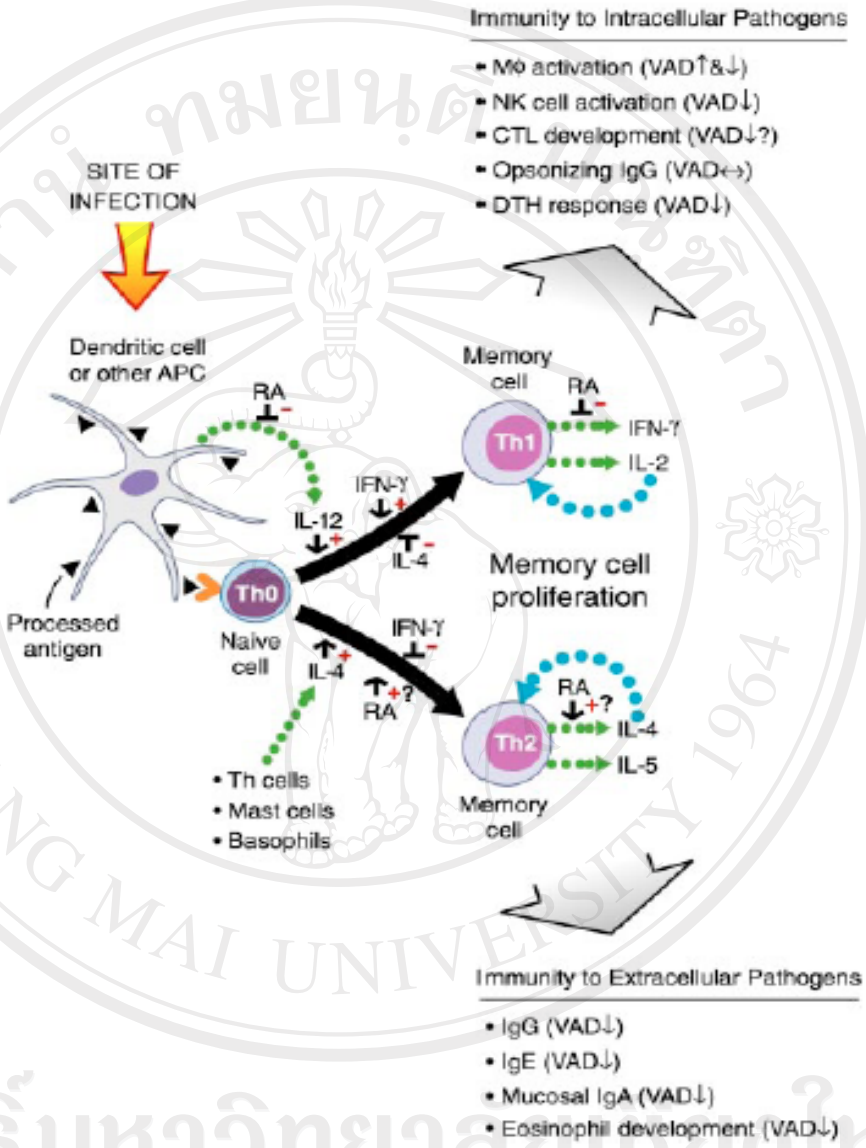


Figure 2.6 Effects of vitamin A deficiency and of retinoic acid on cytokine production on mechanisms of immunity

- = block; + = stimulates; ↑ = enhance; ↓ = diminished; ↔ = not changed

RA = retinoic acid

(Source: Vitamin A, infection, and immune function. Annu. Rev. Nutr.2001; 21: 167-192)

Micronutrient deficiency may be produced by infectious diseases in 5 ways; first, by decreasing food intake (anorexia); second, by impairing nutrient absorption; third, by causing direct nutrients losses; fourth, by increasing metabolic requirements or catabolic losses; and fifth, by impairing utilization e.g. by impairing transport to target tissue (Figure 2.7) [38, 41].

HIV is a viral infection. Many studies examined the association of low serum retinol concentrations and HIV severity or progression of disease [42, 43]. Most data of the association of vitamin A status and severity of HIV infection suffers from the flaw that serum retinol concentrations are used to identify "deficiency" subjects. However, short term interventions in better-nourished adults' populations in the United States demonstrated no improvement in disease or immune function parameter [41].

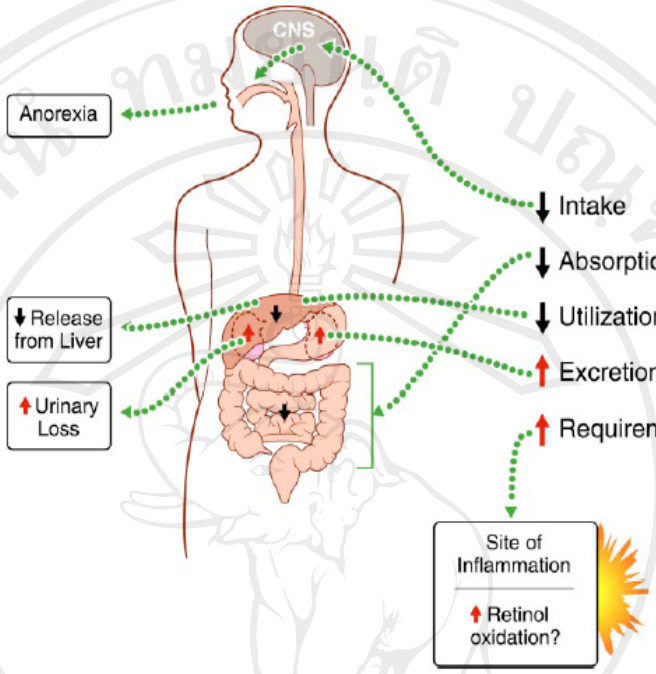


Figure 2.7 Three mechanisms by which infections are known to impair vitamin A status: decreased intake due to anorexia; decreased absorption; and increase urinary excretion. Infection mat also affect vitamin A status by impairing utilization of retinol stores in the liver, by diminishing transport from stores to peripheral tissues, or by increasing requirements (e.g. by increasing oxidative degradation of retinol at sites of inflammation)

(Source: Vitamin A, infection and immune function. Annu. Rev. Nutr.2001; 21: 167-192)

2.4.2 Vitamin E

2.4.2.1 General information of vitamin E

Vitamin E is a potent antioxidant and has an ability to modulate host immune functions. It is a mixture of several related compounds known as tocopherols which the tocopherols are comprised of 4 forms (α, β, λ, and δ). The most commonly distribute and active form of vitamin E is α-tocopherol, as shown in Figure 2.8. It is absorbed from the intestines. The current RDA for α-tocopherol suggests 15 mg/day for adults. It is stored mainly in the fatty (adipose) tissues, the liver and muscles [36].

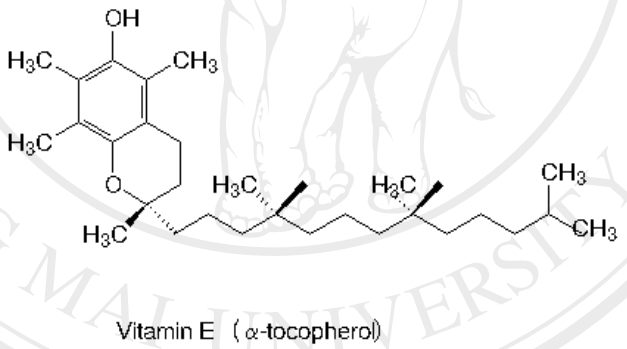


Figure 2.8 Structure of α-tocopherol

2.4.2.2 Role of vitamin E as an antioxidant in HIV infection

Vitamin E is a powerful antioxidant, protecting cellular membranes and as a free radical scavenger by blocking the peroxidation of polyunsaturated fatty acids (PUFA). In the absence of vitamin E, oxidation of PUFA is generated and readily propagated to numerous other PUFA along the membrane resulting in cell damage. Vitamin E is an important nutrient in the function of the immune system. Vitamin E deficiency induces the impairment of both humoral and cellular immunity. Not only it affect the function and integrity of membrane lipids, protein and nucleic acids, but it also can regulate signal transduction and gene expression which are associated with depress production of antibodies by B lymphocyte, reduce T-cell proliferation on stimulation and an increased rate infection [44] .

In HIV-infected patients, oxidative stress is elevated which resulting in promoting replication. In addition, ROS promote the dissociation of NF-κB from I-κB and subsequently increase the production of NF-κB. Vitamin E is also a potent antioxidant and decreases the production of tumor necrosis factor-alpha (TNF-α) which enhances the production of ROS and subsequently increases the expression of NF-κB (Figure 2.9). Furthermore, vitamin E may directly and indirectly inhibit the promotion of ROS and thus can inhibit the development of HIV/AIDS [44].

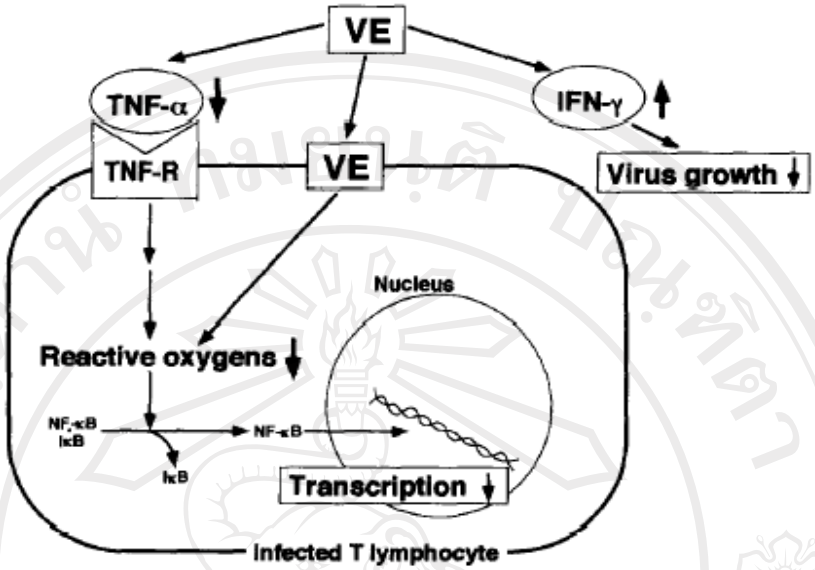


Figure 2.9 The improvement of immune dysfunction by vitamin E.

VE = vitamin E; ↓ = decrease; ↑ = increase

(Source: vitamin E and immunity. Vitam Horm. 2000; 59:305-336)

2.4.3 Vitamin B12

2.4.3.1 General information of vitamin B12

Vitamin B12 is cobalt -containing compounds and the structure is shown in Figure 2.10. Vitamin B12 is the largest water soluble vitamin. Plant foods do not normally contain vitamin B12, so humans typically obtain vitamin B12 from animal foods. The main source foods of vitamin B12 are beef, milk, shellfish, organ meats and highly fortified cereals. The RDA for vitamin B12 is 2.4 µg per day for adults [45]. In general, vitamin B12 intakes in adults are adequate but in the older individual approximately 10 -30% developed vitamin B12 deficiency [36].

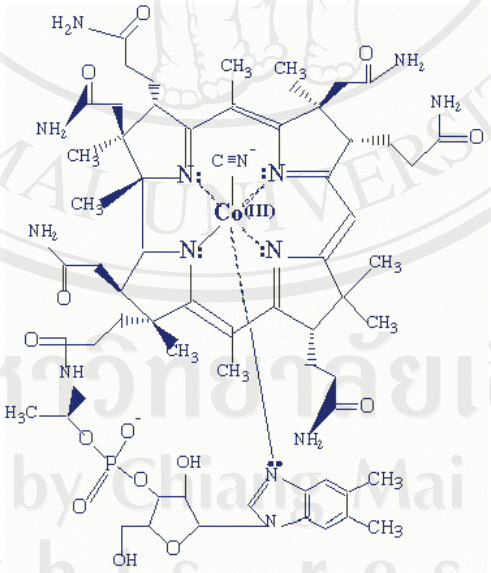


Figure 2.10 Structure of vitamin B12

2.4.3.2 Role of vitamin B12 on immune function in HIV infection

Vitamin B12 in human body is a cofactor for 2 enzymes in human. One enzyme converts homocysteine to methionine and converts L - methylmalonyl - CoA to succinyl - CoA. These 2 reactions are critical for normal DNA synthesis and subsequent cell proliferation, especially in blood formation and neurological function [46, 47]. The details of vitamin B12 functions are as follow;

Cofactor for methionine synthase

Methylcobalamin is required for the function of the folate - dependent enzyme, methionine synthase. This enzyme is required for the synthesis of the amino acid, methionine from homocysteine. Methionine is required for the synthesis of S - adenosylmethionine (a methyl group donor used in many biological methylation reactions including the methylation of a number of sites within DNA and RNA). Inadequate function of methionine synthase can lead to an accumulation of homocysteine, as shown in Figure 2.11.

Cofactor for L - methylmalonyl - CoA mutase

Vitamin B12 also is required by the enzyme that catalyzes the conversion of L - methylmalonyl - CoA to succinyl - CoA. This biochemical reaction plays an important role in the production of energy from fats and proteins. Methylmalonyl - CoA is formed as an intermediate in the conversion of valine, isoleucine, methionine and threonine to succinyl - CoA which required vitamin B12 as a co - factor. Succinyl - CoA is also required for the synthesis of hemoglobin. Figure 2.12 shows diagram of

metabolism of methylmalonyl CoA. Vitamin B12 deficiency results in the accumulation of methylmalonic acid and lead to neurological dysfunction.

Vitamin B12 deficiency leads to reduce numbers of CD4+ and CD8+ cells suppress natural killer cell (NK cell) activity which causes increased susceptibility to infection [48]. Previous studies indicated that HIV-infected patients increased demand for vitamins. Immune system activation leads to increase formation of ROS which deplete antioxidants including vitamin B12 and methyltetrahydrofolate [48, 49].

Vitamin B12 deficiency in HIV/AIDS patients may be due to malabsorption from opportunistic bowel infections or from HIV- associated enteropathy and due to decrease intrinsic factor secretion [50, 51]. Vitamin B12 deficiency may occur in up to 23% of HIV-infected patients who did not receive HAART [52]. Furthermore, development of vitamin B12 deficiency is significantly associated with a decrease in CD4+ T cell count and progression to AIDS and also decreased viral load. Treatment with HAART may down-regulate an over activated immune system in the patients and antioxidant status may improve. Thus, vitamin B12 might also increase, even without supplementation [53, 54].

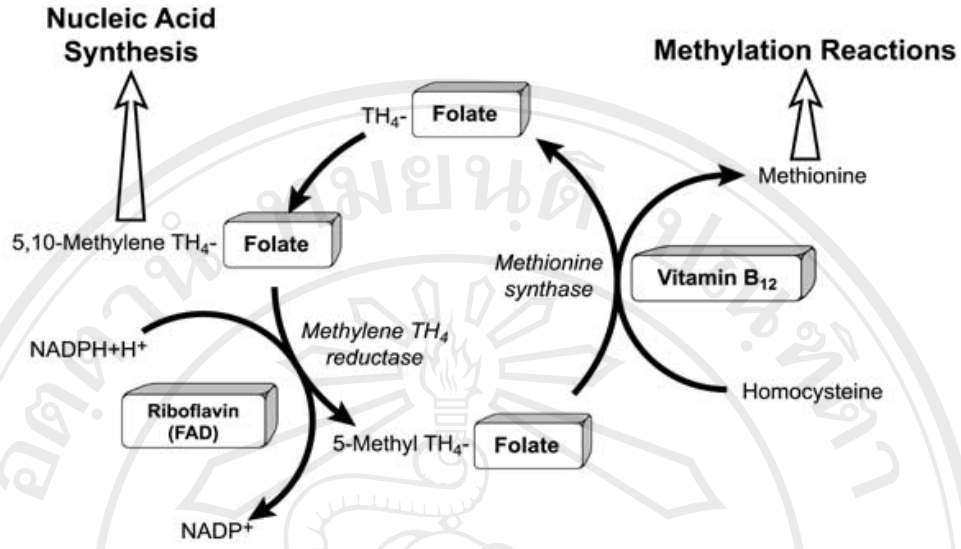


Figure 2.11 Metabolism of methionine

(Source: Nitrous oxide induced elevation of plasma homocysteine and methylmalonic acid levels and their clinical implications. JIACM. 2005; 6(1):48-52)

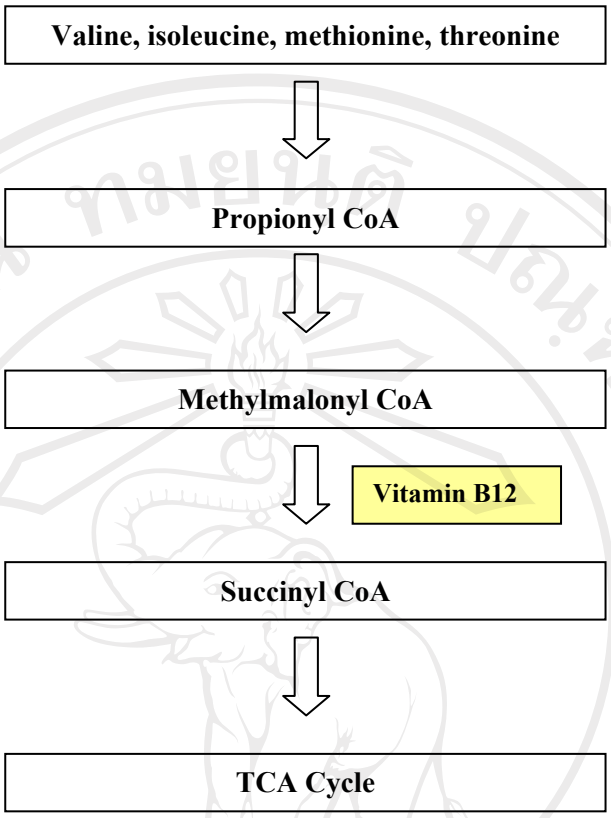


Figure 2.12 Metabolism of methylmalonyl CoA.

(Source: Nitrous oxide induced elevation of plasma homocysteine and methylmalonic acid levels and their clinical implications. JIACM. 2005; 6(1):48-52)

2.4.4 Zinc

2.4.4.1 General information of zinc

Zinc promotes antioxidant and immune functions, stabilizes and maintains the structural integrity of biological membranes, and plays a pivotal role in skin and connective tissue metabolism and repair. Zinc is an integral constituent of a large number of enzymes including antioxidant enzymes, and hormones including glucagons, insulin, growth hormone and sex hormones. Recommended dietary allowances (RDAs) for zinc is 11 mg/day for male and 8 mg/day for female. It is present in meat and other protein foodstuffs, but intestinal absorption is affected by other dietary constituents. Absorbed zinc enters the liver where it is incorporated into zinc metalloenzymes and exported to peripheral tissue in plasma, bound to albumin. The total plasma zinc concentration of 12-25 μmol/L, over 90% is associated with albumin, <10% with alpha-2 macroglobulin, and a small amount, <1%, complexes to amino acids and other low molecular weight species. Zinc homeostasis is achieved by regulation of enter hepatic re-circulation. An amount of zinc equivalent to the total absorbed zinc is re-excreted into the gut in intestinal fluids. In normal health person, zinc output by the gut is equal to the total dietary intake. Urinary excretion of zinc is low (around 10 μmol/day), and does not vary markedly with dietary supply. It is increased in catabolic states, by certain drugs and/or chelating agents [36].

2.4.4.2 Role of zinc as an antioxidant in HIV infection

The ability of zinc for retarding oxidative processes involves two mechanisms. The first mechanism involves in the protection of sulfhydryl groups against oxidation, as shown in Figure 2.13. The second mechanism, involves in the prevention of $HO^{\bullet}$ and $O_2^{\bullet-}$ production [55].

The effect of zinc deficiency on the primary antioxidant system can be divided into enzymatic and nonenzymatic components such as vitamin E, glutathione, metallothionein. Zinc deficiency also effects on the gene expression and enzymatic activity e.g. extracellular SOD and DNAase, as shown in Figure 2.14. Therefore, zinc deficiency on HIV-infected host immune cells influence to free radical excess and may stimulate increasing of HIV replication [55, 56].

Zinc is essential for HIV replication but there is no published evidence that increasing zinc concentration in vitro stimulates HIV replication. In vitro experiment showed that zinc concentration of 100 μg/mL (100-fold usual plasma concentration) inhibited HIV-RNA transcription without being toxic to the host cell. Zinc deficiency in humans also affects the cytokine production of T-helper cell 1 (Th1) lead to an imbalance between Th1 and Th2. Therefore, zinc is important factor for the efficiency of the immune system in HIV infection, in particular for CD4+ T cell growth and function. Effect of the strong depletion of CD4+ T cells in HIV infection lead to the appearance of opportunistic infections [57 - 59].

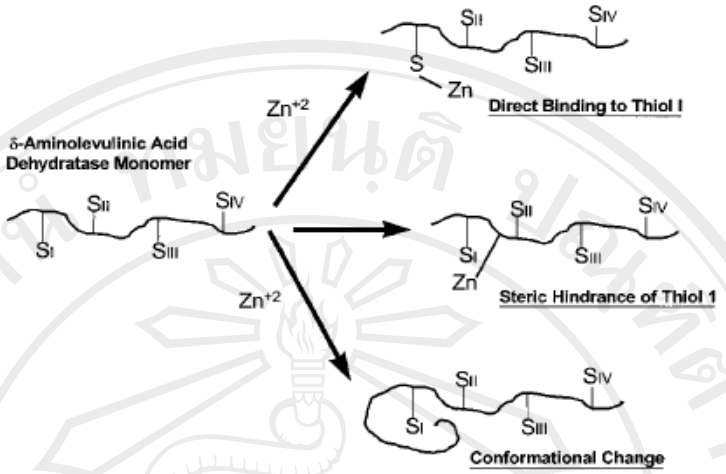


Figure 2.13 Propose mechanisms of zinc stabilization of sulfhydryl groups in enzyme δ-aminolevulinic acid dehydratase

(Source: The antioxidant properties of zinc. Presented at the international workshop the national institutes of health in Bethesda, MD, on November, 1998)

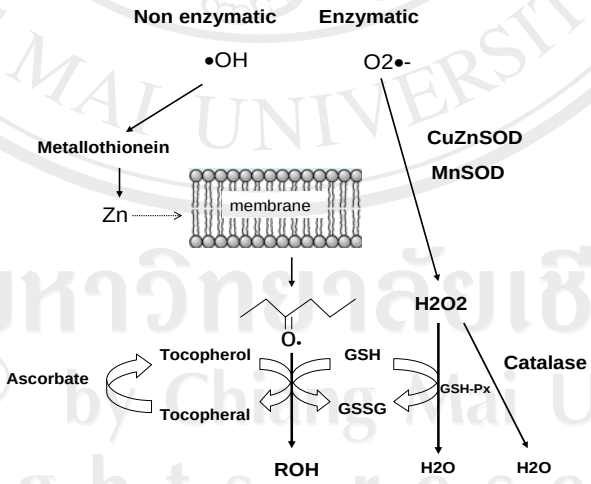


Figure 2.14 Enzymatic and non enzymatic free radical defense system

(Source: Free Radical and Radiation Biology Program. The University of Iowa. Iowa City. 2003)

2.4.5 Selenium

2.4.5.1 General information of selenium

Selenium is essential to good health but required only in small amounts. It is incorporated into proteins to make selenoproteins which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals [60, 61]. RDA for selenium in adults is 70 μg for male and 55 μg for female [62]**.** Several forms of selenium enter the body as part of amino acids within proteins and the two most common forms are selenomethionine and selenocysteine which are found mainly in plants and animals respectively [63]. The primary sites of absorption are from throughout the duodenum. Selenium absorption is not affected by body selenium status. Absorption of selenium is closely related to multiple nutritional factors that inhibit or promote absorption. Vitamins A, C, and E along with reduced glutathione enhance absorption of the element. In contrast, heavy metals (i.e. mercury) decrease absorption via precipitation and chelating. Selenium is excreted via two main paths: urinary (50-67%) and fecal (40-50%). High intakes of selenium can lead to ventilatory elimination of the mineral in the form of dimethylselenide [64].

2.4.5.2 Role of selenium as an antioxidant in HIV infection

Selenium has an important role in both immunologic function and antioxidant defense. Evidence presented suggests that an imbalance between diminished host antioxidant defense and increased oxygen radicals and pro-inflammatory cytokines

create oxidative stress in HIV infection. Selenium acts as an integral constituent of the antioxidant enzyme glutathione peroxidase (GSH-Px) which catalyses the destruction of hydrogen peroxide (H_2O_2) and organic lipid peroxides [65, 66]. On the other hand, GSH has been show to suppress the nuclear transcription factor kappa B (NF- kB) mediated HIV pro-viral transcription. In addition, selenium has importance to activated T cell function and it may enhance resistance to infection via a more efficient Th1/Th2 response. Selenium deficiency in HIV infection is associated with glutathione peroxidase (GPx) activity and immune dysfunction, including impaired phagocytic function [66].

2.5 Micronutrients status in various populations

2.5.1 Micronutrients status in healthy population

Several studies reported the level of serum or plasma vitamin A and zinc in different age group in various countries (Table 2.6 to Table 2.7). In Thailand, Boonsiri et al. [67] established baseline data for serum vitamin A and zinc concentrations of the healthy adults aged 23-75 years in the northeast region. The mean value of serum vitamin A was 0.659 ± 0.201 μg/mL (95%CI; 0.645-0.673) which is the range reported within from other countries (Table 2.6). The mean of serum zinc levels for Thai healthy individuals showed 1.19 ± 0.22 μg/mL (95%CI; 1.18-1.20). It seems to be higher than the other places and no significant difference of the zinc levels between males and females, the results show in Table 2.7.

Table 2.6 Vitamin A levels in serum or plasma of people from various countries

Country	Age (years)	Specimen	Mean ± SD of vitamin A levels [a] (μg/mL)	
			Males (n)	Females (n)
Germany (Berlin)	22-41	Plasma	-	0.476 ± 0.106 (77)
Greece	> 65	Plasma	0.410 ± 0.149 (82)	0.381± 0.149 (118)
Finland	20 -64	Plasma	0.774 (427)	0.639 (366)
China	35-64	Plasma	0.510± 0.100 (25)	0.410 ± 0.074 (25)
Japan	7-86	Serum	0.785 (618)	0.682 (1196)
Vietnam	40-59	Serum	0.811± 0.258 (32)	0.690 ± 0.241(68)
(Medium income)			0.831± 0.301(40)	0.619 ± 0.221(58)
(Low income)			0.690 ± 0.249 (39)	0.521± 0.180 (59)
Laos	Adults	Plasma	0.407 ± 0.060 (14M/F)	-
Thailand (Bangkok)	16-60	Serum	0.851[b] (14)	0.851[b] (58)
Thailand (Northeast)	20-35	Serum	0.776 ± 0.221 (34)	0.564 ± 0.175 (51)
	36-50		0.745 ± 0.201 (232)	0.570± 0.158 (287)
	51-75		0.742 ± 0.198 (78)	0.662 ± 0.186 (62)
Arab	18-63	Serum	0.504 ± 0.06	
Saudi Arabia	6-18	Serum	> 0.201 (500M/F)	
Algeria	ND	Serum		0.401± 0.120 (250)
North Cameroon	3-61	Serum	0.152± 0.009 (40M/41F)	
USA (pre-menopause) (Post- menopause on HRT) (Pre-menopause on HRT)	40-70	Plasma		0.507± 0.049 0.547± 0.029 0.610± 0.029 (39)[c]

M = male, F = female, HRT = hormone replacement therapy, ND = no data.
[a] Vitamin A level calculated in μg/mL unit (284.5 μg/mL = 1 μmol/L).
[b] Median.
[c] Number of total subjects.

Table 2.7 Zinc levels in serum or plasma of people from various countries

Country	Age (years)	Specimen	Mean ± SD of zinc level [a] (μg/mL)	
			Males (n)	Females (n)
Spain (Canary islands)	6-75	Serum	1.18 ± 0.49 (187)	1.14 ± 0.55 (208)
(Tarragona)	16-65	Serum	1.06 (196)	1.23 (185)
(Toledo)	25 – 40	Serum	0.87(5 M/10F)	-
Germany	22 – 75	Plasma	1.08(68M/F)	-
Norway	20 – 54	Serum	Range 0.89 -1.18 (200 M/F)	-
Italy	37.8 [b]	Serum	0.90 ± 0.20 (508 M/F)	-
Northern Ireland (year 1983 – 1984) (year 1989)	25-64 21.6 (M)[b] 22.1 (F)[b] 78.3(M)[b] 88.6 (F)[b]	Serum	0.79 ± 0.11(1144) 0.86 ± 0.14(1142) 1.13 ± 0.16 (30) 0.98 ± 0.20 (31)	0.76 ± 0.09(1055) 0.83 ± 0.13(1034) 1.00 ± 0.16 (32) 0.93 ± 0.18 (33)
UK (London)	19-58	Plasma	0.95 ± 0.13 (36)	0.96 ± 0.08 (31)
India	ND	Serum	1.01± 0.35 (ND)	-
Thailand (Bangkok)	20-80	Serum	0.83 ± 0.01(121)	0.83 ± 0.01 (191)
Thailand (Northeast)	20-35 36-50 51-75	Serum	1.24 ± 0.19 (68) 1.19 ± 0.21(434) 1.17± 0.22 (161)	1.26 ± 0.21 (94) 1.18 ± 0.22 (298) 1.19 ± 0.24(57)
Japan	30-49 50-69 >70		-	0.92 ± 0.12 (21) 0.84 ± 0.16 (5) 0.85 ± 0.16 (4)
Venezuela	ND	Serum	0.84 ± 0.20 (444M/43F)	-
USA (Oklahoma)	50(M)[b] 38.4(F)[b]	Plasma	0.96(204)	0.88 (54)

M = male, F = female, HRT = hormone replacement therapy, ND = no data
[a] Vitamin A level calculated in μg/mL unit (284.5 μg/mL = 1 μmol/L).
[b] Median, [c] Number of total subjects.

The study of Viroonudomphol et al. [68] examined vitamin A and vitamin E status in Thai volunteers who attended the outpatient Department, General Practice Section, Rajvithi Hospital, Bangkok. The population comprised overweight (BMI ≥ 25.0 kg/m^2) and normal (BMI = 18.5-24.9 kg/m^2). The results showed that the median serum retinol concentration in overweight subjects was 0.80 μg/mL (rang 0.15-1.31 μg/mL) compared with 0.85 μg/mL (range 0.34-1.17 μg/mL) in control subjects (p = 0.0736). The median serum α-tocopherol concentration in overweight subjects was 7.45 μg/mL (range 2.71-12.34 μg/mL) compared with 8.08 μg/mL (range 2.28-13.04 μg/mL) in control subjects ($p<0.05$). Prevalence deficiency of retinol was found 2.8% (< 0.20 μg/mL) and 11.1% of α-tocopherol (< 7.0 μg/mL) in obese subjects. Serum retinol deficiency did not appear in control subjects but 25% of serum α-tocopherol deficiency was found in control group.

The study of Songchitsomboon and Komindr [69] established the reference range of serum zinc of healthy adults, living in Bangkok and surrounding districts and the effects of sex, age, BMI, smoking and drinking habit on serum zinc. This result showed that serum zinc concentration was 0.83 ± 0.01 μg/mL (range 0.53-1.12 μg/mL), while there was no significant difference in serum zinc concentrations between males (0.83 ± 0.01 μg/mL, range 0.54-1.12 μg/mL) and females (0.53-1.12 μg/mL). Serum zinc concentrations of the same samples classified by age showed that serum zinc was significantly lower in subjects aged 60-80 years than the younger age groups. Zinc concentration in serum with respect to different influencing factors of BMI, smoking and drinking habits. However, no differences were observed for serum zinc concentration in these factors.

The other study of Songchitsomboon et al. [70] was conducted to evaluate serum zinc level in 118 patients (53 females and 65 males) admitted to the medical ward of Ramathibodi Hospital, Bangkok, and compare with 312 healthy adults living in Bangkok and surrounding districts. The mean concentration of zinc in healthy adults was 0.83 ± 0.15 μg/mL (mean ± SD) when compared with serum zinc of patients, significantly decreased serum zinc concentrations were found in patients with gastrointestinal and hepatic (0.60 ± 0.07 μg/mL, $p < 0.01$, n = 13), infectious (0.61 ± 0.04, $p < 0.001$, n = 30), renal (0.61 ± 0.04, $p < 0.001$, n = 14), cardiovascular (0.68 ± 0.05, $p < 0.005$, n = 22)and malignant diseases (0.61 ± 0.08, $p < 0.001$, n = 9).

An examination for serum selenium concentration of healthy people in Miyagi, Japan found that the mean serum selenium concentrations (± SD) for male and female were 111.0 ± 0.9 μg/L and 102.5 ± 0.7 μg/L, respectively [71].

The national university of Singapore heart study was a cross sectional survey of a random sample of 941 persons aged 30 to 69 years from general population of Singapore which examines ethic differences in blood vitamin A, C, E and selenium. Results show in table as follow [72];

	Mean (95% confidence intervals)		
	Vitamin A (μg/mL)	Vitamin E (μg/mL)	Selenium (μg/L)
Indians (I) - Males, n =166 - Females, n =166	0.66 (0.64,0.68) 0.51 (0.49,0.53)	12.9 (12.4,13.4) 12.8(12.3,13.3)	117 (115,119) 115 (113,117)
Malays (M) - Males, n =144 - Females, n =142	0.67 (0.65,0.69) 0.54 (0.52,0.56)	13.6 (13.0,14.2) 13.3 (12.7,13.9)	122 (120,124) 122 (120,124)
Chinese(C) - Males, n =158 - Females, n= 165	0.68 (0.66,0.70) 0.52 (0.50,0.54)	12.6 (12.0,13.2) 12.6 (12.0,13.1)	126 (124,128) 119 (117,121)

2.5.2 Micronutrients status in HIV-infected patients in pre-HAART

2.5.2.1 Micronutrients status in HIV-infected patients

Micronutrients play a critical role in the proper functioning of the immune system. Therefore, it is not surprising that HIV-positive individuals have presented with low serum levels of many micronutrients.

A cross-sectional study of Baeten et al. [73] conducted to examine the relations between vitamin A deficiency (serum retinol < 0.30 μg/mL) and HIV status, HIV disease stage and marker of the acute phase response (C-reactive protein, CRP and α_1-acid glycoprotein, AGP). The subjects were 400 HIV-infected and 200 HIV-uninfected women in Mombasa, Kenya. The results showed that HIV status was strongly related to vitamin A deficiency and the acute phase response. HIV-infected women had lower serum vitamin A concentrations (median vitamin A concentration was 0.274 μg/mL, range 0.028-0.81 μg/mL) than HIV-negative females (0.365 μg/mL, range 0.15-0.785 μg/mL). Including, HIV-infected women had higher serum CRP and AGP concentrations than HIV-negative females. Furthermore, 59% of the HIV-seropositive females had serum vitamin A levels deficiency compared with 29% of HIV-seronegative females (OR = 3.5, 95% CI: 2.4-5.2). The prevalence of an acute phase response (CRP ≥ 10 mg/mL and /or AGP ≥ 1.2 g/L) was 44% among HIV-seropositive females and 14% among HIV-seronegative females (OR = 4.7, 95% CI:3.0-7.5). In addition, there was a moderately strong and statistically negative correlation between serum vitamin A concentration and the concentration of CRP (r = - 0.45, p< 0.001) and AGP (r = - 0.43, p< 0.001). 55% of those with vitamin A

deficiency also had an acute phase response compared with 27% of those who were not vitamin A deficiency. Because of HIV status and the acute phase response were both related to vitamin A deficiency. In this analysis, HIV infection and the acute phase response were independently associated with vitamin A deficiency (OR = 2.7, 95%CI: 1.9-4.0; OR = 2.8, 95% CI: 1.9-4.1, respectively). However, there were no statistically significant association between vitamin A deficiency and the acute phase response among HIV-uninfected women (OR = 1.5, 95%CI: 0.6-3.7). Therefore, females who were both HIV infected and had an acute phase response were at a particularly high risk of vitamin A deficiency. Furthermore, the result showed vitamin A deficiency was associated with higher viral load and lower CD4+ T cell count. Serum of vitamin A levels were significantly correlated with both of these markers of HIV disease severity ($r = -0.29$, $p < 0.001$ for HIV-plasma viral load; $r = 0.24$, $p < 0.001$ for CD4 level). Finally, this study found that supplementation was associated with significantly higher serum vitamin A concentrations among those with no acute phase response, although serum concentrations did not rise to non deficient levels within this subgroup.

Beach et al. [52] determined the prevalence of specific nutritional abnormalities in HIV-1 infected homosexual men in USA who were asymptomatic. The mean serum vitamin A level in HIV-seropositive subjects (0.44 ± 0.10 μg/mL) was within normal limits (0.30-0.60 μg/mL), but significantly lower than HIV-seronegative subjects (0.52 ± 0.11 μg/mL) ($p < 0.05$). In contrast, no difference in mean serum vitamin E levels was observed between the two groups (8.9 ± 4.8 μg/mL and 9.8 ± 4.7 μg/mL for HIV-seropositive and HIV-seronegative subjects, respectively) and these levels also were within normal limits (6-15 μg/mL) in both groups. Mean of vitamin B12 in

HIV- seropositve subjects (390 ± 200 pg/mL) was not different from those observed in HIV-1 seronegative controls (434 ± 155 pg/mL), normal range for vitamin B12 was defined as 241-700 pg/mL. Mean level of zinc was not significantly different and was within normal limit in both HIV-seropositive (0.85 ± 0.17 μg/mL) and HIV-seronegative (0.87 ± 0.14 μg/mL) subjects. However, the mean value was very near the lower limit of the range considered to be normal. There was a significantly higher prevalence of low blood levels of micronutrients in the asymptomatic HIV-infected subjects compared with HIV-seronegative controls, vitamin A (11% & 0%), E (19% & 0%), B12 (11% & 0%) and zinc (21% & 17%), respectively.

Tang et al. [74] study examined the association between serum vitamin A and vitamin E levels and risk of progression to three key outcomes in HIV-1 infection: first AIDS diagnosis, CD4+ T cell decline to < 200 × 10^6/L and mortality. Serum vitamin A and vitamin E were measured at the enrollment visit of 311 HIV-infected homo-/bisexual men participating in the Baltimore/Washington DC. The mean age of the study population was 34 years (range 30-65 years) and the mean of CD4+ T cell counts were 643 ± 314 cells × 10^6/L with only 11 men (4%) have CD4+ T cell counts below 200 cells × 10^6/L. Although most of the men (81%) were in the asymptomatic stage of their infection, 55% had elevated serum CRP levels (≥ 8.0 mg/L) and 23% had low serum albumin levels (< 35 g/L). The mean and median levels of serum vitamin A were 0.75 ± 0.31 μg/mL and 0.70 μg/mL, respectively. The mean and median of vitamin E were 8.36 ± 5.04 μg/mL and 7.41 μg/mL, respectively. These mean and median levels were within the normal range for both vitamins. 31 subjects (10%) had serum retinol levels below 0.40 μg/mL and only 7 subjects (2%) had serum

retinol levels below 0.30 μg/mL and 22% of subjects had serum vitamin E level deficiency(< 5.0 μg/mL). However, 29% of subjects had total intakes of vitamin A (including vitamin supplements) below the recommended dietary allowance (RDA) and 27% had total vitamin E intakes below the RDA. Univariate analysis in this study showed that subjects with vitamin E deficient were more likely to be non-whites (p = 0.02), not have a college degree (p = 0.007) and not used antiretroviral drugs before the onset of AIDS (p = 0.07). Cox proportional hazards models for serum micronutrient levels and time to first AIDS diagnosis showed that no significant difference was observed in risk of AIDS progression between deficiency and adequate serum vitamin E levels. However, men in the highest quartile of serum vitamin E level (>10.12 μg/mL) showed a 34% decrease in risk of progression to AIDS compared with those in the lowest quartile after this model adjusted for the following covariates [relative hazard (RH), 0.67; 95%CI 0.45-0.98].

In Africa, Visser et al. [75] demonstrated that retinol levels of HIV-infected adults were low in 39% of patients in asymptomatic stage and 48% of symptomatic stage and 79% of patients with clinical stage III and stage IV, respectively. The median retinol level for patients with early disease was 0.341 μg/mL, compared with 0.306 and 0.238 μg/mL for those with stage III and IV disease, respectively (p < 0.01). In terms of plasma zinc, the median level for patients with early disease was 0.843 μg/mL, compared with 0.778 μg/mL and 0.719 μg/mL for those with stage III and IV disease respectively (p < 0.01). In addition, there were weak positive associations between CD4+ T cell count and plasma levels of retinol (r = 0.27) and zinc (r = 0.31), using the Spearman correlation coefficient. Furthermore, multivariate analysis demonstrated that stage IV disease was independently associated with a threefold

increased risk of low plasma retinol levels (<0.30 μg/mL) after adjusting for CD4+ T cell count, hemoglobin levels and body weight.

Baum et al.[53] examined levels of nutrition status of vitamin A, E, C, B1, B2, B6, B12 (cobalamin), folate, zinc, iron, plasma proteins and immune function CD4+ T cell count and β2M (β2–micro globulin) in HIV seropositive homosexual men. Four evaluations were conducted at 6 months intervals over 18 months (baseline, 6, 12, 18 months). The results demonstrated that development of deficiency of vitamin A and vitamin B12 were associated with a decline in CD4+ T cell count, while normalization of vitamin A, vitamin B12 and zinc were associated with higher CD4+ T cell count. For vitamin B12 low baseline status significantly accelerated HIV disease progression determined by CD4+ T cell count.

Bogden et al. [76] studied a cross-sectional study of 106 HIV-infected and 29 uninfected subjects in Newark to assess relation between nutrient concentrations and CD4+ T lymphocyte counts and comparison of nutrient concentrations in stage of infection which HIV-infected subjects were classified into Centers for Disease Control and Prevention (CDC) stages A, B, C on the basis of the presence of opportunistic infections. The results showed that the relations between CD4+ T cell count and plasma zinc concentration ($r = 0.21$, $p = 0.044$) and stage of infection did not influence mean plasma zinc significantly. However, the lowest zinc concentrations occurred in stage-C subjects: 36% had concentrations below the normal range of 0.70-1.20 μg/mL. The percentages of uninfected and stage-A and -B subjects with value < 0.70 μg/mL were 14.3%, 14.7% and 30.2%, respectively. These percentages showed a significant increase in below-normal zinc concentrations with infection and increasing disease severity (chi-square test for trend, $p < 0.05$).

Including, the results reported that significantly lower plasma zinc concentrations were found in subjects who reported loss of appetite (0.72 ± 0.04 μg/mL compared with 0.86 ± 0.03μg/mL) or vomiting (0.69 ± 0.03μg/mL compared with 0.84 ± 0.03μg/mL) than subjects who did not.

A cross-sectional study of Look et al. [77] determined serum selenium in 104 HIV-infected patients (83 outpatients and 21 patients with ongoing AIDS defining events). The patients were classified into three stages of the disease, I, II, III according to the 1993 CDC classification system of HIV infection. GSH-Px activities, plasma SH and plasma GSH concentrations were determined in a subset of 24 patients at stage I and 12 patients at stage III with active AIDS-defining disease. The results showed that mean serum selenium levels were lower in CDC stage II (68.7 ± 20.9 μg/L; $p< 0.01$; n = 34) and stage III (51.4 ± 14.7 μg/L; $p < 0.01$; n = 37) than healthy subjects (89.2 ± 20.9 μg/L; n = 72) and stage I patients (82.3 ± 20.5 μg/L; n = 33). Serum selenium and GSH-Px activity in AIDS patients were significantly lower than asymptomatic patients and healthy subjects, where plasma SH and GSH concentrations were lower in both, asymptomatic and AIDS patients than in the controls. In addition, serum levels were positively correlated with CD4+ T cell count ($r = 0.42$; $p < 0.001$; n = 104). Thus, this study concluded that stages I-III of HIV disease were characterized by significant impairments of anti-oxidative defenses provided by selenium, GSH-Px, SH-groups.

2.5.2.2 Micronutrients supplement in HIV-infected patient

The study of Jiamton et al.[13] was conducted to examine the impact of high dose multiple micronutrient supplementation on survival and disease progression among HIV-infected individuals in Thailand. The participants were 41 and 81 HIV-infected males and females adults living in Bangkok. They had not been taking micronutrients or antiretroviral in the last 30 days and had a CD4+ T cell counts in the range 50×10^6 - 550×10^6 /L. They were randomized to receive micronutrients or placebo for a period of 48 weeks. The results showed that at baseline measurements, mean of plasma selenium was 126.34 ± 15.79 μg/L and vitamin E was 9.48 ± 3.88 μg/mL. At the final follow up, plasma levels of vitamin E and selenium were significantly higher than in the placebo group; the differences in the increasing mean from baseline were 4.61 and 2.63 μg/L, respectively. In addition, this results found that the mortality rate was significant lower in the micronutrients arm among those whose CD4+ T cell counts were either $< 200 \times 10^6$ /L or 100×10^6 /L, the hazard ratios being 0.37 (95%CI, 0.13-1.06; p = 0.052) and 0.26 (95%CI, 0.07-0.97; p = 0.03) respectively. There was no significant difference in mortality rates between the micronutrients and placebo groups among trial participants with higher CD4+ T cell counts.

Tang et al. [74] study reported that there was no significant association between serum vitamin A levels and risk of progression to AIDS. Data concerning food and supplemental intake of vitamin A and E were available for 271 of the 311 subjects. Men who reported current use of multivitamin or single vitamin E supplements had significantly higher serum tocopherol levels than those who were not taking supplements (p = 0.0001). Serum retinol levels were unrelated to intake multivitamin

or single vitamin A supplements. These data suggested that high serum levels of vitamin E may be associated with slower HIV-1 disease progression, but no relationship was observed between retinol levels and disease progression in this population who had repletion of vitamin A.

2.5.3 Micronutrients status in HIV-infected patients receiving HAART

Most of the studies documenting low serum micronutrient status and HIV disease progression are conducted prior to the initiation of HAART therapy and may not accurately reflect the micronutrient status of the HIV-positive patients seen today. More recent studies compare serum micronutrient status before and after initiation of HAART.

2.5.3.1 Micronutrients status in HIV-infected patients receiving HAART

Tang et al. [78] conducted a study to examine serum antioxidant level in 175 HIV-positive and 210 HIV-negative injecting drug users (IDUs) in Baltimore, Maryland. At the time of data collection, 30 of the HIV-positive IDUs were receiving antiretroviral therapies (ART) including protease inhibitor (PI), 43 ART without PI, 22 monotherapy, and 80 not on any ART. Serum antioxidant levels were examined according to HIV status, CD4+ T cell counts among the HIV-positive study subjects (< 200, 200-499 and ≥ 500cells/μL) and antiretroviral therapy (ART) regimens. The results showed that median serum α-tocopherol levels were significant higher in HIV-positive study subjects (0.744 μg/mL) than in HIV-negative study subjects

(0.712 μg/mL, p = 0.04) but serum retinol levels were not significantly different between two groups, 0.373 and 0.383 μg/mL for HIV-negative study and HIV-positive study subjects, respectively. Among the HIV-positive study subjects, no differences were observed by categories of CD4+ T cell counts. For the latter, HIV-positive study subjects were divided into four treatment categories consist of 1) no ART, 2) nucleoside analogue monotherapy, 3) combination therapy without PIs and 4) combination therapy with PIs. In univariate analyses base on median, α-tocopherol significantly different among the treatment groups. Furthermore, results from multivariate ANOVA models showed that serum levels of α-tocopherol remained significantly higher in the PI group than in the other three groups combined, even after adjusting for dietary intake, supplement intake, gender, injection drug use, alcohol intake and cigarette smoking. Another possibility is that serum levels of these vitamin are higher in the PI group because they are lipid soluble and hyperlipidemia and hypercholesterolemia have been recently reported in HIV-infected individuals on ART regimens containing PIs. In particular, serum α-tocopherol level has been showed to be highly correlated with serum cholesterol and total lipid concentrations in healthy adults. Although Tang A. M. et al. were unable to measure total lipid levels in their population, they did obtain serum cholesterol levels of their study subjects and serum α-tocopherol was significantly correlated with serum cholesterol levels (r = 0.57, p = 0.0001). In addition, explanation for this finding is that there is a reduction of oxidative stress in patient on PI therapies.

Study of Jones et al [79] determined the prevalence of low serum retinol, α-tocopherol, zinc and selenium concentrations in HIV-infected subjects taking HAART and to assess the association of micronutrient levels with HIV disease status. Results showed micronutrient levels deficiency by gender, 5% of males and 14% of females had low retinol, 8% of males and 3% of females had low selenium and 7% of males and no females had low α-tocopherol, 40% of males and 36% of females had low zinc. Associations of micronutrient quartiles with HIV disease status (CD4+ T cell count) were analyzed using multivariate linear regression. Age, race, poverty, years HIV-positive, history of intravenous drug use (IDU) ever, BMI, and liver score were include in all multivariate models for continuous dependent covariates. The results showed that no micronutrient was associated with CD4+ T cell count - related disease status in multivariate models. In males, only zinc showed a trend for difference in disease status by quartiles. Log viral load was lower among men in the upper 3 zinc quartiles (658, 773, 984 μg/L) compared with men in the lowest quartile; this difference approached significance for zinc in quartile 3. The high prevalence of low serum zinc in this study was consistent with in pre-HAART studies. Previous their study, an acute-phase response (CRP) is an unlikely cause of low serum zinc. Their participants had few active infections, and serum zinc levels were not associated with CRP. However, low serum zinc has been associated with chronic diarrhea, and 16% of males and 12 % of females reported current diarrhea (≥ 30 days) at the time of their visit. The association of lower log viral load with higher serum zinc in this study could reflect improvement of immune status and improved virologic control because of higher zinc.

Baum et al. [80] examined the impact of specific micronutrient deficiency in combination with immune function and using of antiretroviral treatment (zidovudine) in HIV-1 infected males and female. The 125 HIV sero-positive participants in this study were seen every 6 months in a community-based study clinic for 3.5 years. Blood was evaluated for immune function and biochemical evaluation of nutrition status that included vitamin A, E, B6, B12, zinc and selenium. Plasma vitamin A and E were determined by HPLC. Levels below 0.3 μg/ml and < 5 μg/ml for vitamin A and E, respectively were considered inadequate. Plasma vitamin B12 levels were established by radioisotope dilution assay and deficiency was defined as plasma levels < 200 pg/ml. An enzymatic assay was used to determine vitamin B6 status, expressed as an activity coefficient (AC), with an AC >1.85 considered to be evidence of deficiency. The results demonstrated that deficiency of vitamin A, B12, zinc and selenium were significantly associated with mortality. Regarding, the relation between nutritional deficiencies and survival, the risk ratio for vitamin A, vitamin B12, zinc, selenium deficiency were 3.2 ($p < 0.03$), 8.3 ($p < 0.009$), zinc 2.9 ($p < 0.04$) and 19.9 ($p < 0.0001$), respectively (at CD4+ T cell count < 200 cell/mm^3 and CD4+ T cell time-dependent counts). However, neither vitamin B6 nor vitamin E deficiency was associated with increased risk mortality.

A longitudinal study of Hepburn et al. [81] was to examine vitamin B12 and folate levels HIV-infected patients from the Infectious Disease Clinic at Brooke Army Medical Centre. The effect of antiretroviral therapy on vitamin B12 was studies on subset of patients that serum vitamin B12 levels were recorded before and after the initiation of therapy. Vitamin B12 measurements within 6 months of initiating therapy and within 12 months after starting therapy were included. There were 38 patients

with available vitamin B12 measurements before and after the initiation of antiretroviral therapy. There was a significantly increase in vitamin B12 levels after antiretroviral therapy was initiated (416 & 535 pg/mL, $p = 0.04$ using pair t-test). Among these patients with vitamin B12 levels pre- and post-antiretroviral therapy, in which multivitamin use was recorded, 58% were taking multivitamin prior to initiating therapy and 65% were taking multivitamins after therapy was started. However, results from the regression models indicated that factors independently associated with vitamin B12 levels were ethnicity ($\beta = 0.196$, $p = 0.003$) and higher folate levels ($\beta = 0.02$, $p < 0.001$) and years since HIV diagnosis ($\beta = 0.01$, $p = 0.099$) was nearly significant. For visits in which a low serum vitamin B12 was measured, CD4+ T cell counts were significantly lower (435 and 550 cells/mm^3, $p = 0.02$) compared to patient visits with normal vitamin B12 measurements, whereas other clinical parameters were not statistically different. Including, vitamin B12 deficiency were not associated with anemia or neurologic symptoms. However, patients with vitamin B12 were more likely to have thrush.

Remacha et al. [21] evaluated the prevalence of vitamin B12 deficiency in 126 HIV-infected patients receiving HAART. The result showed that the prevalence of vitamin B12 deficiency was significantly lower in patients receiving HAART than in historical patients who did not receive HAART (8.7% & 28%). Conclusion, the prevalence of vitamin B 12 deficiency decreased after the introduction of HAART.

Rousseau et al. [19] examined the micronutrients of 44 HIV-positive patients, mostly drug users, before and after the initiation of HAART. HAART involved taking two nucleosides inhibitors of reverse transcriptase and at least one protease inhibitor. The first nutritional evaluation was conducted in 1995 before HAART, the second

was conducted in 1998 when patients received HAART. The results showed that before receiving HAART, 77% of patients and 22.7% had low plasma selenium (mean 51.5 μg/L) and zinc (mean 79 μmol/L) levels, respectively. When a comparison between patients in the group with CD4+ T cell count < 250/mm^3 and patients in the group with CD4+ T cell count > 250/mm^3 the both groups revealed significantly lower levels of plasma selenium ($p = 0.048$) whereas no significant difference for zinc concentration. However, women with CD4+ T cell count < 250/mm^3 showed zinc concentration significantly lower than in men ($p = 0.019$). On the other hand, selenium plasma concentrations in males were significant lower in females of the same group ($p = 0.042$). No significantly differences were note for other micronutrients. Three years later after receiving HAART, mean of selenium level was 93.9 μg/L, 10% of patients had low selenium values. Mean of zinc level was 71.2 μmol/L, 26.6% of patients had low zinc values. There were no significant differences in mean concentration of selenium and zinc compared with the pre-HAART, neither for selenium nor for zinc matched with sex. Patients treated with HAART had practically no weight loss. Although patients who did not receive HAART had good immune status, the mean weight loss was up to 4.6 kg. Thus, HAART reduces selenium and zinc deficiencies and may help avoid weight loss independently of the CD4+ T cell count.

Mocchegiani et al. [20] studied to associate CD4+ T cell counts and plasma zinc levels in HIV-infected patients with receiving HAART. Subjects consisted of a total of intravenous drug-user HIV subjects, mean age 28 ± 5.5 (range 20-36 years). They were recruited with CD4+ T cell counts between 250-400 cell/mm^3 and were received HAART, 2 nucleotide analogues (AZT + 3TC) and 1 PI (IND or RIT).

Immunological and nutrition parameters were detected at months 0 and 4 after HAART therapy as well as possible opportunistic infections censoring. Relative risk factors were evaluated form 0 to 4 of observation. The data of relative risk factors were compared with HIV-infected subjects with CD4+ cell counts between 250 and 400cell/mm^3 treated with only AZT because of no availability of other antiviral drugs in this study. Control groups were healthy young subjects, mean age 25 ± 3.4 years. The results showed that at time 0, zinc level was 0.78 ± 0.06 μg/mL, CD4+ T cell counts were 350 ± 38 cells/mm^3 and found that CD4+ T cell counts and body weight (Δ%) were reduced at time 0 as compared to young healthy controls ($p < 0.001$). After 4 months ago, HAART increases zinc levels, body weight and CD4+ T cell counts and reduced HIV-RNA as compared to time 0. In addition, HAART increased CD4+ T levels and zinc concentrations which were both inversely correlated with HIV-RNA ($r = 0.72$; $p < 0.01$, $r = - 0.57$; $p<0.05$, respectively) in HAART treated HIV subjects.

The cohort study of Lai et al.[82] evaluated the associations between plasma zinc levels and mortality in 121 HIV-positive homosexual males, Florida, U.S.A. Plasma zinc levels were measured at baseline and then at semiannual visits. Zinc deficiency was defined as 0.75 μg/mL. HIV related deaths were confirmed by review of death certificates. Cox proportional hazards regression models were performed to evaluate the effects of CD4+ cell counts, antiretroviral therapy, zinc deficiency and plasma zinc levels on HIV related mortality. The variables were significantly associated with elevated mortality: CD4+ T cell counts < 200 cells/mm^3. (relative risk [RR] = 16.55: 95%CI 4.71-58.21], zinc deficiency (RR = 5.30; 95%CI 1.42-19.72]. Conversely, high plasma zinc levels (RR = 0.95: 95%CI 0.93-0.99] seemed to be beneficial to survival. Antiretroviral therapy was marginally associated with an increased mortality

[RR = 2.46; 95%CI 0.98-6.18]. In addition, multivariate analyses Cox regression models were used to determine whether plasma zinc had an independent effect on survival, after adjustment for potential confounding factors, such as low CD4+ T cell count (< 200 cells/mm^3) and antiretroviral therapy, there still remained a 5-fold increase in mortality for study subjects with plasma zinc deficiency (RR = 4.98; 95%CI 1.30-19.00).

2.5.3.2 Micronutrients supplement in HIV-infected patients receiving HAART

The study of Junior et al. [83] evaluated the benefits of supplementation with 800 mg/day of α-tocopherol with regard to cellular viability in HIV-1 seropositive patients undergoing anti-retroviral therapy. Participating in this study were 29 voluntary patient with average age of 30 years (rang 21-38 years). These patients had not previously been administered antiretroviral medication. Among the patients, 15 comprised the control group who received antiretroviral therapy plus placebo, and 14 patients comprised the study group who received anti-retroviral therapy plus a supplement of 800 mg/day vitamin α-tocopherol. The patients provided sample before the treatment commenced and after 60, 120, and 180 days. The percentage of viable lymphocytes showed significant increase as a consequence of treatment time in both groups studied (p = 0.0002). There were also a significantly difference between treatments in terms of the percentage of viable lymphocytes overtime, with the study group under antiretroviral therapy and supplement with α-tocopherol presented higher levels of viable lymphocytes (2.49 fold increased) at 180 days of treatment compared

to the control group under antiretroviral therapy with placebo (1.95 fold increased). Determination of serum levels of α-tocopherol, the baseline serum levels of α-tocopherol were similar in the control and supplement groups (6.72 ± 2.11 μg/mL vs. 8.10 ± 1.90 μg/mL) and the supplemented group showed significant increase in serum levels of α-tocopherol after 60 days of treatment. In addition, the plasma levels of HIV-RNA showed significant decrease as a consequence of treatment time in the groups studies ($p = 0.0001$). After 120 days of treatment, a significant decrease on average, compared to the baseline and the level observed after 60 days of treatment was verified. Furthermore, the CD4+ T cell count demonstrated a significant increase as a consequence of antiretroviral therapy for the two groups studied ($p = 0.0002$), without differences between the group supplemented with α-tocopherol and control group. Patients of both groups after 60 days of treatment presented a significant increase in the CD4+ T cell count relation to the respective baseline averages (1.32 fold for the control group and 1.52 fold for the study group).