CHAPTER II

LITERATURE REVIEW

2.1 HIV Infection

2.1.1 Pathophysiology of HIV Infection

The human immunodeficiency virus (HIV) is the etiology of HIV infection and acquired immune deficiency syndrome (AIDS). Any HIV-infected individual with a CD4+ T cell count of <200/μL has AIDS by definition regardless the presence of symptoms or opportunistic infections. HIV is transmitted by sexual contact, by blood and blood products, and by infected mothers to her infant either perinatally or via lactation.[6]

HIV is a retrovirus which is capable to transform its genomic RNA into DNA by the reverse transcriptase enzyme. The replication cycle of HIV begins with the binding of HIV's gp120 binding protein located on the virus surface and the receptor on CD4 molecule. A gp41 molecule will be penetrating the plasma membrane and of the target cell and then coiling upon itself to bring the virion and target cell together. A preintegration complex composed of viral RNA and viral enzymes is released into the cytoplasm of the target cell.[6]

The preintegration complex will reach the nucleus and promote the reverse transcryption of the genomic RNA into DNA and the protein coat of the complex will be releasing the resulting double strand DNA. In this process, the viral genome is vulnerable to cellular factors that block the progression of infection.

The viral DNA will be integrated into the its host's chromosome by the action of integrase enzyme.[6]

It is known that a number of mechanisms responsible for cellular depletion of CD4 T cells can be induced by direct infection and destruction by HIV. The combination of viral pathogenic and immunopathogenic events that occur during HIV infection through the development of advanced stage diseases is complex and varied.[6]

2.1.2 Antiretroviral Therapy

Suppression of HIV replication is an important component in prolonging life as well as in improving the quality of life in patients with HIV infection. Currently available drugs for HIV infection are divided into 4 classes: reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, and fusion inhibitors. The reverse transcriptase inhibitors class includes nucleoside reverse transcriptase inhibitors (NRTI), nucleotide reverse transcriptase inhibitors (NtRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI).[6]

The antiretroviral drugs should be used in combination regimens to avoid drug resistance. Patients initiating antiretroviral therapy (ART) must be willing to commit life-long treatment.

Table 2. Indications for ART initiation in HIV-infected patients[6]

No	Indications
1.	Acute infection syndrome
2.	Chronic infection A. Symptomatic disease (including (HIV-associated nephropathy) B. Symptomatic disease 1. CD4+ T cell count < 500 µL 2. Pregnancy
3.	Postexposure prophylaxis

2.2 Stavudine Administration in HIV-Infected Patients

2.2.2 Pharmacology of Stavudine

Stavudine or 2'.3'-didehydro-3'deoxythimidine (d4T) belongs to the NRTI class drugs. The NRTIs act by competitive inhibiting HIV reverse transcriptase. The incorporation of the drug into the growing viral DNA chain may cause premature chain termination due to inhibition of binding with the incoming nucleotide. All agents in the NRTI class require intracytoplasmic activation via phosphorylation by cellular enzymes to their triphosphate form. Stavudine is excreted by active tubular secretion and glomerular filtration. The serum half life is 1.1 hours and the intracellular half life is 3.0-3.5 hours. Stavudine has high oral bioavailability (86%) that is not food dependent.[13]

Table 3. The pharmacological characteristics of stavudine[13]

Agent	Class of Agents	Recommended Adult Dosage	Characteristic Adverse Effects	Comments
Stavudine	NRTI	30-40 mg bid, depending on weight	Peripheral neuropathy, lipodystrophy, hyperlipidemia, rapidly progressive ascending neuromuscular weakness (rare), pancreatitis	Avoid concurrent zidovudine and neuropathic drugs (e.g. ddI, zalcitabine, isoniazid)

2.2.2 Toxicity of Stavudine

The NRTI class drugs have several adverse effects that are associated with mitochondrial toxicity such as lactic acidosis, neuropathy, pancreatitis, hepatic steatosis and lipodystrophy. The nucleoside analogue linked most strongly to lipoatrophy is stavudine, particularly when used together with didanosine (ddI). Stavudine-associated lipodystrophy may give rise to severe insulin resistance and many components of metabolic syndromes in HIV-infected patients.[14]

The antiviral activity and toxicity of stavudine is mainly dependent on its active metabolite form or stavudine triphosphate (d4T-TP). A polymorphism of thymidylate synthase gene was found to have association with d4T-TP intracellular level and thus the development of lipodystrophy syndrome. The level of d4T-TP is strongly affected by the dose received by patients, the activity of nucleoside transporters, and the cellular activation state.[15,16]

2.2.3 Stavudine Phasing-Out

Stavudine has been used for long period in Indonesia and other developing countries because the drug does not need initial laboratory test to start the therapy and its cost is lower compared to other NRTIs such as zidovudine (AZT), tenofovir (TDF) and abacavir (ABC). The drug is distributed in combination with lamivudine (3TC) and nevirapine (NVP). Many countries have implemented the plan to switch stavudine with tenofovir or zidovudine but insufficient financial issues have slowed the pace of phase-out.[11]

According to The National Guidance of Clinical Management in HIV Infection and Antiretroviral Therapy in Adults and Adolescents published in 2011, the initiation of ART in ART naïve patients should be started when the CD4 count reaches less than 350 cells/mm^3. The starting regimen should be using tenofovir as the first line while stavudine is phased out due to its known toxicity. The previous guidance published in 2007 still recommending stavudine as first line regimen combined with lamivudine and either efavirenz (EFV) or nevirapine. Stavudine is also replaced by tenofovir in treatment for HIV infection coinfected with tubercolusis.[17]

Due to its toxicity the government of Indonesia recommended health care provider to initiate ART with zidovudine or tenofovir to ART naïve patients and alter the drug to patient who had received stavudine after 6 months of usage without any clinical side effect shown.[14,17]

2.3 Lipodystrophy in HIV-Infected Patients

2.3.1 Definition of Lipodystrophy

A variety of metabolic disorders are seen in the context of HIV infection. These might be a direct consequence of HIV infection, related to ART side effects, or secondary to neoplasms and opportunistic infections. It is known that between 33 and 75% of patients taking ART are developing a syndrome often called lipodystrophy syndrome. The 'lipodystrophy' term was introduced as 'a complex medical condition including an apparent abnormal fat redistribution and metabolic disturbances in HIV patients receiving Protease Inhibitors (PIs) therapy.' [1]

Lipodystrophy syndrome is characterized by elevation in plasma triglycerides, total cholesterol, and apolipoprotein B, as well as hyperinsulinemia and hyperlipidemia. The risk of the syndrome increases with the duration of treatment, patient's age and the level of immunodeficiency. This syndrome may harbor a significant risk of developing cardiac disease such as atherosclerotic vascular disease and myocardial infarction. There is an increased relative risk of myocardial infarction after 7 years of ART exposure.[1,6]

Lipodystrophy syndrome physically appears as characterized body shape changes associated with fat redistribution, which mostly includes truncal obesity and peripheral wasting. Truncal obesity is characterized by an excessive abdominal fat compared with peripheral fat, a dorsocervical fat pad or 'buffalo hump' and enlargement of the breasts. The peripheral wasting or lipoatrophy is characterized by Bichat's fat pad in the cheek, fat loss in facial and buttock

regions, as well as the prominence of the veins in the legs. These changes may develop at any time ranging from 6 weeks to several years after the initiation of ART.[6,12,18]

Lipodystrophy is frequently seen in patients receiving regimens containing NRTI class drugs and PI class drugs, although almost all antiretroviral combinations are associated with fat redistribution. It has been suggested that lipoatrophy changes are particularly severe in patients taking NRTI class drugs, especially thymidine analogue such as stavudine and zidovudine. Lipoatrophy was observed no less than one year after the initiation of ART. The current recommendations of lipodystrophy syndrome include switching stavudine or zidovudine to abacavir or tenofovir. The other recommendations are dietary changes and lifestyle modifications.[1,6]

2.3.2 Diagnosis of Lipodystrophy

The diagnosis of lipodystrophy often set on clinical practice based on individual interpretation rather than evaluated classification. This is caused by the absence of a consensus on a case definition of lipodystrophy in HIV. In most cases, peripheral lipoatrophy is diagnosed when there is a significant fat loss or about 30% of peripheral fat. In clinical settings, lipodystrophy is usually diagnosed from the occurrence of apparent clinical signs or patients reporting them.[1]

A lipodystrophy severity grading scale (LSGS) is an assessment method to visually diagnose lipodystrophy. The body parts assessed in LSGS are face, arms,

buttocks, legs, abdomen, neck, and breasts. The score is the mean of the sum of scores given by patients and the physician for both fat loss and fat accumulation. The diagnosis of lipodystrophy is set with an overall score > 7 or there are severe fat changes in ≥ 1 body location.[16]

Table 4. Lipodystrophy Severity Grading Score[16]

Degree of fat loss and fat accumulation	Score
Absent	0
Mild (noticeable on close inspection)	1
Moderate (readily noticeable by patients/physician)	2
Severe (readily noticeable by casual observer)	3

Anthropometric measurements are considered in diagnosing lipodystrophy. Several parameters such as waist circumference, sagittal diameter, and skin fold thickness are used in individual long term monitoring. Advanced imaging technique such as dual energy x-ray absorptiometry (DEXA), CT Scan, and MRI may also detect lipodystrophy. DEXA is widely used in epidemiological study although it is unable to distinguish subcutaneous adipose tissue with visceral adipose tissue in truncal obesity.[1]

2.3.3 Pathophysiology of Stavudine-Associated Lipodystrophy

The pathogenesis of lipodystrophy induced by NRTI drugs is different with PI class drugs. Peripheral fat loss is a major symptom observed in NRTI induced lipodystrophy. It is well established that long exposure to NRTIs may cause mitochondrial toxicity. The clinical manifestations of this toxicity are

lipodystrophy, hyperlactatemia, hepatic steatosis and polyneuropathy. Mitochondrial toxicity is tissue specific by means the uptake of NRTIs and their phosphorylation may be different in different cell types.[1]

The mechanism of lipodystrophy has not been well understood. A 'γ-polymerase hypothesis' has been proposed to explain the phenomenon. NRTIs are prodrugs that have to be phosphorylated first to NRTI-triphosphates before they can inhibit the HIV reverse transcriptase. The NRTI-triphosphates also block γ-polymerase activity which is responsible in mitochondrial DNA (mtDNA) replication. The γ-polymerase is essential in maintaining an adequate bioenergetic level for accurate cell function. This inhibition leads to a decline in mtDNA content in human cell. A further effect of this decline is the disturbed respiratory chain, impaired ATP synthesis, and decrease in NADH and FADH consumption in cells. The mtDNA depletion and structural changes in mitochondria will lead to increased rate of apoptosis in subcutaneous adipocyte cells.[1]

Several experiments show that certain pharmacodinamic requirements are needed for NRTI uptake into mitochondria, the phosphorylation and incorporation into DNA. These requirements include thymidine kinase activity and deoxynucleotide transport specifity of the mitochondrial membrane, which is thought to contribute in thymidine analogue such as stavudine-associated lipodystrophy.[1]

Stavudine-associated lipodystrophy is considered to be an inflammation process. The inflammation is marked with increased plasma level of proinflammatory and inflammatory cytokines. The lipodystrophy is also

considered the apoptosis of adipocytes, as evidenced by decreased adipogenic factors. A study identified that there were mtDNA depletion and ultrastructural abnormalities of mitochondria in subjects treated with stavudine. The *in vitro* and *in vivo* analysis demonstrated diminished intracellular lipids, reduced expression of adipogenic transcription factor (peroxisome proliferator-activated receptor γ (PPAR-γ) and sterol regulatory enhancer-binding protein 1 (SREBP-1) and increased apoptotic indices in fat cells.[1,14]

Stavudine is the strongest antiviral agents among its class that is associated with lipodystrophy. Stavudine has been identified as a risk factor of impaired secretion of adiponectin in fat tissue. There are significant differences between stavudine compared with other NRTIs in its relative potency to interact with γ-polymerase. A study showed that switching stavudine to other NRTIs may cause an increase in mtDNA count and a decrease in apoptotic indices.[1,14]

Stavudine associated-lipodystrophy is also affected by duration of treatment. A study found that each month of stavudine use, the risk of lipodystrophy increased by 2%. This is thought to be a result from increased duration of mitochondrial toxicity.[14]

Another study found that HLA-B*4001 gene found in major histocompability complex (MHC) may play a role in stavudine-associated lipodystrophy. Patients with HLA-B*4001 is more vulnerable from lipodystrophy compared to patients with no HLA-B*4001. This genetic factor is indeed stronger than the duration of treatment.[14]

2.4 Dyslipidemia in HIV-infected Patients

2.4.1 Definition of Dyslipidemia

Dyslipidemia is a primary major risk factor of coronary artery disease (CAD) and may be occurring before the other major risk factors are present. Epidemiologic studies concluded that hypercholesterolemia and coronary atherosclerosis are risk factors for ischemic stroke.[19]

Dyslipidemia in HIV-infected patients may be seen as a part of lipodystrophy syndrome. Hypertrigliceridemia and hypercholesterolemia are the most common lipid abnormality found several weeks after ART initiation. The characteristic of dyslipidemia in HIV-infected patients receiving ART includes elevated level of total cholesterol (TC), Low Density Lipoprotein-cholesterol (LDL-c), tryglicerides (TG) and decreased High Density Lipoprotein-cholesterol (HDL-c). Stavudine and PI class drugs increase the blood levels of TC, LDL-c, and TGs with variable effects on levels of HDL-c.[11,20]

A study discovered that there were some decreases in TC, HDL-c, and LDL-c levels in patients at the time of HIV infection before treatment. By the initiation of ART, TC and LDL-c increased to their pre-infection levels but low HDL-c level persisted. Various classes of ART are known to cause increased systemic very low density lipoprotein cholesterol (VLDL-c) and TG in circulation, a shift to TG-rich VLDL in blood, increased systemic apolipoprotein C-II and apolipoprotein E levels, increased accumulation of CD36-dependent cholesterol ester in macrophages, decreased degradation of lipoprotein B and

impaired fibrinolysis. It is reported that there was a significant increase in VLDL-c level after a short duration of ART.[12,21]

It is recommended to measure fasting lipid levels annually before the initiation of ART and within a or two months after any change in the ART, together with familial history of dyslipidemia or diabetes and alcohol or drugs that alter lipid levels consumption assessment. Traditional risk factor such as age, sex and body mass index (BMI) may also affect the lipid profile's changes after ART initiation.

2.4.2 Diagnosis of Dyslipidemia

Several studies found that non-fasting lipid profiles were not sufficient to identify patients at risk of vascular diseases. To ensure the most precise lipid profile assessment, a fasting lipid profile is now recommended to all patients. A 9-12 hours of fasting is necessary to avoid the effect of food intake toward chylomicron and VLDL-c. According to the US National Cholesterol Education Program, dyslipidemia was defined as TG $\geq$ 150 mg/dL or TC $\geq$ 200 mg/dL or LDL-c $\geq$ 130 mg/dL or HDL-c $\leq$ 40 mg/dL. [11,19]

Table 5. Optimal, borderline, and high risk lipid profile concentration[19]

Lipid, units	Optimal/near-optimal serum concentration	Borderline serum concentration	High risk/very-high risk serum concentration
TC, mg/dL	<200	200-239	≥240
HDL-c, mg/dL	≥60 (negative risk factor)	40-59 (men) 50-59 (women)	<40 (men) <50 (women)
LDL-c, mg/dL	<100 (optimal) (100-129 near optimal)	130-159	160-189 (high) ≥190 (very high)
TG, mg/dL	<150	150-199	200-499 (high) ≥500 (very high)

2.4.3 Pathophysiology of Stavudine-associated Dyslipidemia

Hypercholesterolemia in association with low HDL-c and LDL-c levels was commonly described in HIV-infected patients before the treatment with ART. Several studies explained that the contributing factors were increased apolipoprotein E levels, increased hepatic synthesis of VLDL-c and decreased clearance of triglyceride-rich lipoproteins. There was also an evidence of increased macrophages in adipose tissue in lipoatrophic fat leading to dyslipidemia. The direct effect of HIV infection, the presence of acute-phase reactants and circulating cytokines such as interpheron-α may have contribution to dyslipidemia.[7,21-22]

The specific mechanism of thymidine analogue's effect in dyslipidemia is not well understood. It is known that stavudine-based regimens are associated with early increases in patients' TG and TC levels. The main pathogenetic mechanism which nucleoside analogues are thought to contribute to metabolic changes is mitochondrial toxicity. The thymidine analogue is also thought to reduce the clearance of free fatty acids and tryglicerides, alter adipocyte functions,

reduce lipid content, reduce adiponectin and leptin release, and increase reactive oxygen species (ROS) production and monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) release.[1,18,23]

The toxicity of stavudine is mainly dependent on its active form or stavudine triphosphate. It is known that stavudine triphosphate concentration exerts both antiretroviral and toxic effects. The toxic effect is produced by competitive inhibition of γ-polymerase *in vivo* which will lead to the decline of mtDNA in adipocyte cells. Stavudine is known to have the biggest potential to cause hypertrigliceridemia among all NRTIs. [16,24]

A study in Poland reported that increased TG and TC levels were more frequent in patients treated with stavudine and lamivudine combination, regardless of the third drugs used in the regimens.[25]

2.5 Factors Associated with Lipodystrophy and Dyslipidemia

2.5.1 Age

Patients with dyslipidemia and lipodystrophy are generally older. A study noted that the average age of patients with dyslipidemia or lipodystrophy was 40.2 years while the average age of patients without dyslipidemia and lipodystrophy was 38.5 years (P=0.007). Another study supported this finding that older ages correlated with TG and TC level in HIV-infected patients with stavudine administration (P=0.01 and P==<0.001).[11,12]

2.5.2 Sex

A finding in Uganda showed that male patients had a significant association with the prevalence of dyslipidemia and lipodystrophy (P=0.035). Study taken in Tanzania also concluded that males had significant correlation with TG level in blood (P=<0.001) . There was a contradictory result in study taken in Brazil which stated that there was no correlation between sex and dyslipidemia (P=0.084). [11-12,18]

2.5.3 Duration of treatment

There was a report that longer duration of treatment had association with dyslipidemia in patients taking ART. A study found that the duration of antiretroviral treatment significantly increased the prevalence of dyslipidemia and lipodystrophy. Even though the patients received NNRTI-based regimes, a prolonged duration of treatment is proven to increase the prevalence of dyslipidemia. (P=0.001). This finding is also reported by another study where the median duration of treatment was 2.5 years. (P=0.016).[12,18]

2.5.4 CD4 Count

A study demonstrated that most of patients with stavudine-associated lipoditrophy and dyslipidemia had CD4 counts lower than 200 cells/mm^3. (P=0.010) This was thought to have association with the fact that most of patients

initiating ART already had AIDS-related syndrome. Another study showed that CD4 count level had significant correlation with TG level (P=<0.001).[11,12]

2.6 Confounding Variables

2.6.1 Direct Consequence of HIV-Infection

It has been suggested that a great deal of many cardiovascular complications emerged not simply as a complication of ART but due to survival allowing chronic effects of HIV infection which exert several pathologic effects. This finding is supported by the fact that HIV viremia itself or together with immunodefficiency has been shown to associated endothelial dysfunction, hpercoagulability, vascular damage, and inflammation with elevated C-reactive protein (CRP) level. These effects were known to be independent of ART.[7]

2.6.2 Protease Inhibitors Based Regimens Administration

PIs may impair adipocyte differentiation through multiple biochemical interactions with adipocyte proteosomal gene expression systems, down-regulation of cellular retinoic acid binding protein levels (CRABP), SRBP levels, activation of the adipocyte-renin-angiotensin system and adipokine effects, and decrease in PPAR-α and PPAR-γ. These effects may be manifested as adipose hypertrophy, increased TG levels, decreased HDL-c, adipocyte insulin resistance, hypertension, and tendency to type 2 diabetes mellitus. Ritonavir was reported to cause the highest dyslipidemia frequency, due to its potential to inhibit the CYP3A4.[7,22]

2.6.3 History of Metabolic Syndrome

Metabolic syndrome is a collection of metabolic abnormalities contributing in increased risk of cardiovascular disease and diabetes mellitus (DM). The major features of the metabolic syndrome are central obesity, hypertriglyceridemia, low HDL-c level, hyperglycemia and hypertension. HIV-related lipodystrophy may give rise to several components of the metabolic syndrome.[6]

2.6.4 Sedentary Lifestyle

Unhealthy lifestyle such as smoking and lack of physical activity is known to have impact to the increased cardiovascular risk in HIV-infected patients. Patients from urban areas are more likely to have unhealthy lifestyle compared with those from rural areas. Poor diet also has contribution to the increased cardiovascular risk in HIV-infected patients.[11]

2.6.5 Obesity

Obesity is frequently associated with dyslipidemia. The increase of adipocyte mass and decreased insulin sensitivity may lead to impaired lipid metabolism. The increased insulin level promotes fatty acid synthesis in the liver. Increased dietary intake of fat may also lead to increased level of serum VLDL-c and LDL-c .[6]

2.6.6 Cigarette Smoking

Cigarette smoking was proved to have association with metabolic syndrome. There was a dose-dependent effect between numbers of cigarettes smoked and the increase of syndrome progression. Parameters shown to have association with cigarette smoking were increased TG and LDL-c level and abdominal obesity although the exact mechanism was not clear. A study in Ethiopia concluded that history of smoking had an association with lipodystrophy in HIV-infected patients. [26,28]