ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

INSULIN RESISTANCE, DYSLIPIDEMIA AND CARDIOVASCULAR DISEASE RISK IN HIV-1 INFECTED ADULTS RECEIVING PROTEASE INHIBITOR BASED COMBINED ANTIRETROVIRAL THERAPY IN THE ART CLINIC OF TIKUR ANBESSA REFERRAL HOSPITAL.

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Abstract

Treatment with highly active antiretroviral therapy (HAART) has improved the prognosis of patients with AIDS. However, it has also increased the incidence of various metabolic disorders, in particular insulin resistance accompanied by dyslipidaemia, hyperglycaemia and lipodystrophy. This is often predispose to type 2 diabetes and increased mortality from cardiovascular disease which is more common in protease inhibitor based regimen.

This cross sectional study was designed to assess the occurrence of insulin resistance, dyslipidemia and cardiovascular disease risk in HIV-1 infected adults taking protease inhibitor based combined antiretroviral therapy and to compare with those taking NNRTI-based regimen in a total of 134 subjects that contain equal number of cases and controls. Accordingly, variables like age, sex, type of regimen, duration of ART were collected and anthropometric variables and blood pressure was measured. Moreover, biochemical variables like glucose, insulin and lipid profile were determined using standard and calibrated clinical chemistry analyzers along with a parallel control run in a fasting serum sample collected from the patients. HOMA and total cholesterol to HDL ratio as well as triglyceride to HDL ratio were also calculated using a standard formula.

The results revealed an elevated serum triglyceride concentration and a trend toward increase in insulin resistance on patients treated with PI-based regimen (cases), compared to NNRI-based regimen (controls). Insulin resistance was observed in 34.3% of the cases as compared to 28.4% in the controls as assessed by HOMA-IR. HOMA-IR mean values were similar and did not differ significantly between the two groups. Dyslipidemia was also more prevalent among the cases as compared to the controls. Accordingly, 58.2% patients on PI based regimen and 47.8% on NNRTI-based regimen had high cholesterol levels (> 5.1 mmol/L). However, the difference was not statistically significant ($p = 0.226$) and the same is true for their means (210 ± 41versus 200 ± 35, $P = 0.137$)**.** Equally higher percentage of patients had high LDL level (>2.6 mmol/L) on both groups and there was no statistically significant difference in the proportions as well as mean values between the two groups (65.7% versus 64.2%, $p = 0.856$; 109 ± 32 versus 112 ± 31, $p = 0.584$). In this study, it appeared that hypertriglyceridemia (>1.7 mmol/L) affected almost three fourth (74.6%) of the patients on PI-based regimen and 34.3% on NNRTI-based regimen ($p<0.001$). Statistically significant difference was also observed between the mean values of triglyceride (210 ± 41versus 169 ± 124, $p = 0.003$). Similar trends were apparent when comparing the TG/HDL ratio in both groups, with 74.6% of patients on PI-based regimen and 38.8% on NNRTI-based regimen presenting with high TG/HDL ratio (> 3.8) ($p <0.001$). Further aggravating the cardiovascular risk, a significantly higher proportion of patients on PI-based regimen had higher total cholesterol /HDL ratio (61.2%) as compared with those on NNRTI-based regimen, 40.3% ($p=0.016$). Likewise, the mean differences between the two groups were also statistically significant for both TG/HDL and TC/HDL (6.96 ± 4.6 versus 3.98 ± 2.69 and 5.7 ± 1.7 versus 5.0 ± 1.4) with p values 0.026 and <0.001 respectively. Moreover a significantly higher proportion of patients on PI-based regimen were found to have metabolic syndrome as compared to the controls (32.8% versus 17.9%, $P = 0.047$), with 10.4% of patients on PI-based regimen estimated to have an intermediate (10-20%) risk of developing cardiovascular disease in the coming ten years as compared to only 1.5% on NNRTI-based regimen($P = 0.029$)

Taken together, this study concluded that patients on PI- based cART have higher risk of developing insulin resistance and cardiovascular problems.

Key words:. *Protease inhibitors, Insulin resistance, Dyslipidemia, HOMA, Metabolic syndrome*

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ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
cART	Combined antiretroviral therapy
AZT	Zidovudine
BMI	Body mass index
CCR-5	Chemokine receptor 5
CD4	Cluster of differentiation 4
CBV	Combivir
CRABP-1	Cytoplasmic retinoic acid-binding protein type 1
ddI	Didanosine
EFV	Efavirenz
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme linked immunosorbent assay
FDA	Food and Drug Administration
FDRE	Federalist Democratic Republic of Ethiopia
Glucose CAL	Glucose calibrator
GLUT 4	Glucose transporters 4
GOD	Glucose oxidase
HAART	Highly active antiretroviral therapy
HAPCO	HIV/AIDS prevention and control organization
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostasis model assessment- insulin resistance

IGT	Impaired glucose tolerance
IR	Insulin resistance
IRS-1	Insulin receptor ubstrate-1
LDL	Low density lipoprotein
LPV/r	Lopinavir/ritonavir
LRP	LDL-receptor related protein
MOH	Ministry of Health
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non nucleoside reverse transcriptase inhibitors
NVP	Nevirapine
PDK	PIP_3-dependent protein kinase
PIs	Protease inhibitors
PI3-K	Phosphoinositide 3-kinase
PIP_2	Phosphatidyl inositol 4, 5-biphosphate
PIP_3	Phosphatidyl inositol 3, 4, 5-triphosphate
PKB	Protein kinase B
PLHIV	People living with HIV
POD	Peroxidase
PPARγ	Peroxisome proliferator activated receptor gamma
RA	Retinoic acid
RARE	Retioic acid response element
RXR	Retinoid X Receptor
SH2	Src homology 2 domain
TMB	Tetra methyl blue
TDF	Tenofovir

TNF-α	Tumor necrosis factor alpha
UNAIDS	Joint United Nations program for Acquired Immune Deficiency Syndrome
VLDL	Very low density lipoprotein
WHO	World Health Organization
WHR	Weight to height ratio
3TC	Lamuvidine

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1. INTRODUCTION

1.1. Background

The Human Immunodeficiency Virus (HIV) was discovered in 1981(Dennis et al, 2005). All over the globe, the Joint United Nations program for Acquired Immune Deficiency Syndrome (UNAIDS) estimates that there were 34 million [31.6 million–35.2 million] people living with HIV at the end of 2010. Sub Saharan Africa still bears an inordinate share of the global HIV burden with an estimated 22.9 million people living with HIV in the same year, accounting for 68% of the global total (UNAIDS 2011). In Ethiopia an estimated 1.1 million people live with HIV/AIDS and the adult prevalence was expected to be between 1.4 and 2.8 % for the year 2009(FDRE- MOH-HAPCO, 2010).

Treatment strategies were introduced 5 years after the discovery of HIV (Piacenti et al, 2006). Early regimens consisted of one or two drugs and often led to treatment failure. However, Since the advent in 1995 of Highly Active Antiretroviral Therapy (HAART), which consists of at least three agents, a dramatic improvement has been seen in the number of patients attaining undetectable viral loads, improved CD4+ T cell counts and improved survival (Ismail, 2009).

These drugs work as inhibitors which contain six classes including Chemokine Receptor-5 (CCR5) Inhibitors that block the attachment of the HIV virus to the Chemokine Receptor 5 co-recetors, Fusion Inhibitors which inhibit fusion of the viral and host cell membrane and thus inhibit viral entry to the CD4+ T cells, Nucleoside Reverse Transcriptase Inhibitors (NRTIs) which inhibit reverse transcription by binding to viral deoxyribonucleic acid (DNA) and act as DNA chain terminators, Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) that interfere with reverse transcriptase by direct binding to the enzyme, Integrase Inhibitors (IIs) that inhibit integration of proviral DNA to the host cell's DNA and HIV Protease Inhibitors (HPIs) which competitively inhibit the HIV aspartyl protease enzyme there by impairing viral maturation (Figure 1.1). NRTI's commonly form the "backbone" of the antiretroviral therapy cocktail; two NRTI's are usually combined with one medication from either NNRTI's or PI's (Montessori et al, 2004). Unfortunately, besides improving patient prognosis, these drugs also result in metabolic abnormalities like insulin resistance, hyperglycaemia, dyslipidaemia, and lipodystrophy. Consequently, patients are also at

high risk of developing type 2 diabetes mellitus and premature cardiovascular morbidity (David et al, 2007 and Ismail, 2009).

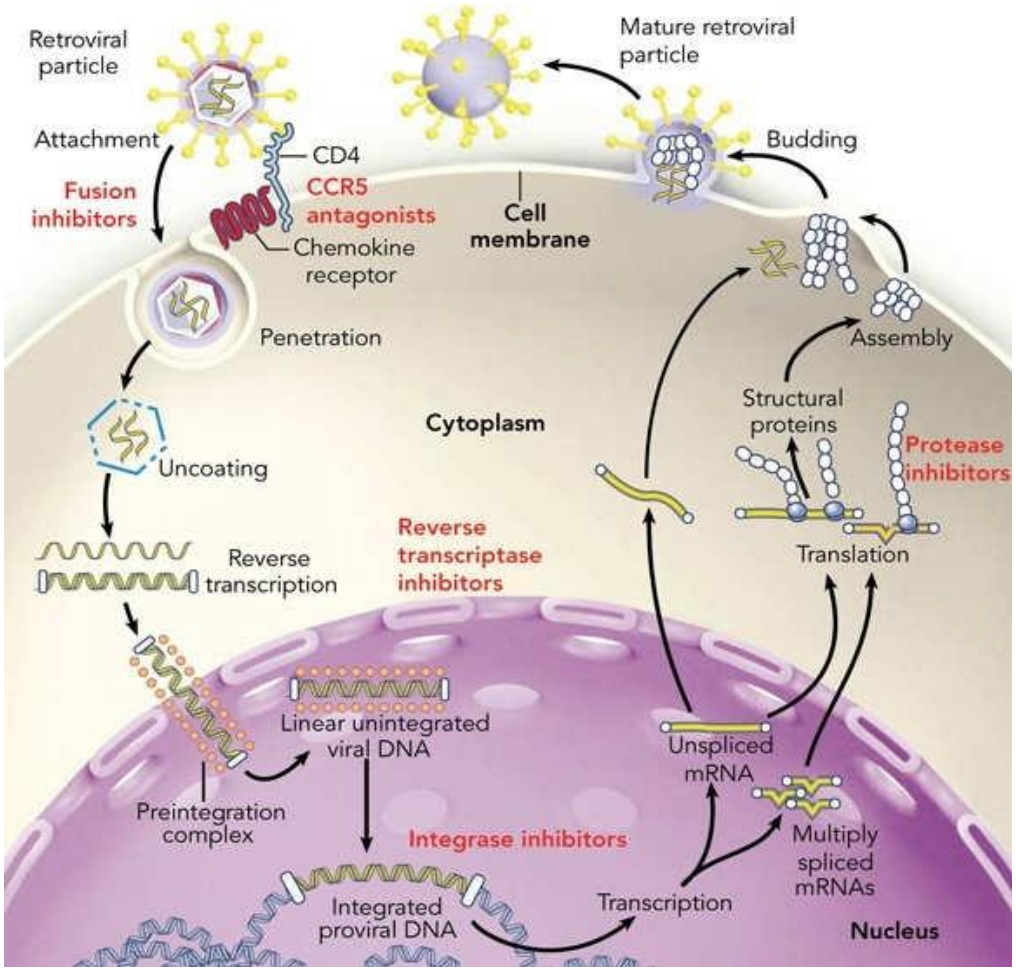

Figure1.1 HIV virus Life cycle and target sites of anti-HIV drugs

(Adopted from an Update on HIV Epidemiology and Antiretroviral Therapy in Hong Kong available at http://www.hksid.org and accessed on November 12, 2011)

Of the ART medications that increase the patients risk to develop insulin resistance and/or diabetes, the most studied and highest risk is associated with the protease inhibitors. Protease inhibitors are the only class of HIV medications that have a direct effect on glucose metabolism. In addition, Use of PIs has been associated with dyslipidemia that is more common and more severe than what was observed before the advent of HAART (Busari et al, 2009; Michael et al, 2003).

Currently there are several types of PIs which include saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir/ritonavir, atazanavir, tipranavir and darunavir. All PIs except atazanavir (ATV) are assumed to cause elevation of total cholesterol, low density lipoprotein cholesterol (LDL-) and triglyceride (TG) and decrease in high density lipoprotein cholesterol (HDL-C), the risk of which is highest for ritonavir (RTV) and Lopinavir/ritonavir and disturbance of lipid profile usually begins in weeks to months after starting therapy (Dube M, 2003). In 1997 the Food and Drug Administration (FDA) put out a class label warning for all PI's that informed physicians of the increased risk for hyperglycemia and diabetes and that close laboratory monitoring for them is warranted. However, later reports claim that use of ritonavir (RTV), lopinavir/ritonavir (LPV/r) and in particular indinavir (IDV) have shown to be more associated with insulin resistance while atazanavir (ATV) does not (Busari et al, 2009; Marie, 2003; Jean, 2004; Friis-Moller et al, 2003 and Hadigam et al, 2000). This implies that insulin resistance induced by protease inhibitors is drug specific.

1.1.1. HIV protease and Protease inhibitors

HIV protease is a homodimeric aspartyl protease with each monomer having 99 amino acid residues. The active site is located at the bottom of a cavity in the dimer interface and is covered by two β-hairpins, called "flaps", one from each monomer that are very flexible, most likely to facilitate substrate binding and product release, and participate in the binding of inhibitors (figure 1.2). The enzyme is responsible for the posttranslational processing of viral polyproteins and subsequent generation of the structural and functional proteins essential for viral replication (Mar´ıa-Jos´e, et al, 2006).

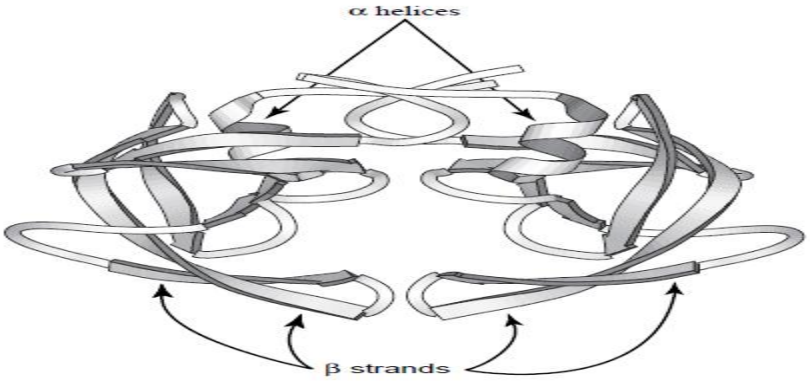

Figure1.2 Structure of HIV-1 protease dimer.

(Adopted from Teacher's Guide and Student Activities for HIV-1 Protease: An Enzyme at Work)

The fact that this step is essential for maturation of the virus and production of infectious particles accounts for the early and current interest in HIV protease as a prime target for antiretroviral drug design. The viral maturation process can be blocked by the use of HIV-1 protease inhibitors, and as a result, the cell releases immature viral particles which are noninfectious (David et al, 1999). As depicted in figure 1.3, protease inhibitors are competitive inhibitors of HIV Protease and almost all are peptidomimetics of the polyprotein cleavage sites (Jana et al, 2009).

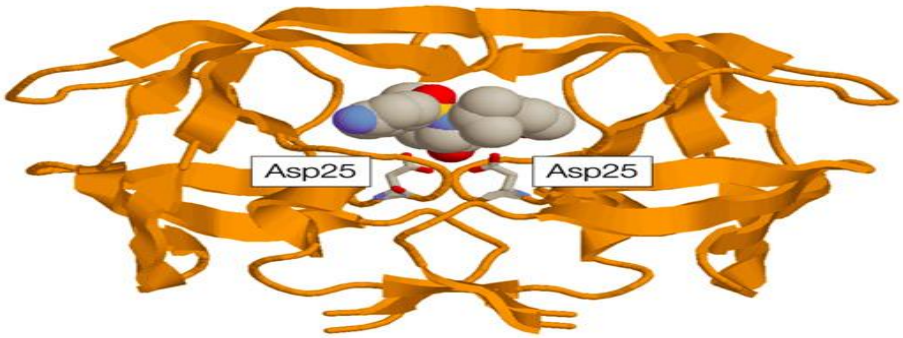

Figure1.3 HIV-1 protease with inhibitor in the active site.

(Adopted from http://www.nature.com/horizon/chemicalspace/background/figs/odyssey_f3.html accessed on June20, 2010)

1.1.2. Insulin signaling

Signaling through the insulin receptor pathway is critical for the regulation of blood glucose levels by insulin. In the post-prandial phase, in response to glucose, the pancreas releases insulin into the bloodstream. Insulin then binds to its receptor. The heterotetrameric (2α- and 2 β-subunits) receptor is a ligand-activated tyrosine kinase (Ismail, 2009).

As shown in figure 1.4, the binding of insulin leads to autophosphorylation of the receptor β-subunit on tyrosine residues. This then activates the intrinsic tyrosine kinase domain and leads to tyrosine phosphorylation of its substrate, Insulin Receptor Substrate-1 (IRS-1). Typically, phosphotyrosine residues interact with a particular type of protein domain, Src Homology 2 (SH2) domain with high specificity and affinity. The tyrosine phosphorylated IRS-1 binds to the SH2 domain of Phosphoinositide 3-Kinase (PI3-K) leading to activation of this enzyme and

conversion of Phosphatidylinositol 4, 5-biphosphate (PIP2) into Phosphatidylinositol 3,4,5-triphosphate (PIP3). The bound PIP3 causes translocation of both PIP3-dependent protein kinase (PDK) and Protein Kinase B (PKB), also known as Akt. This then allows PDK to phosphorylate and then activate PKB. The activation of PKB/Akt is necessary for the final steps leading to glucose transport. This then results in the migration of glucose transporter 4 (GLUT4) from the cytoplasm to the cell membrane to facilitate the uptake of extracellular glucose (Ismail, 2009).

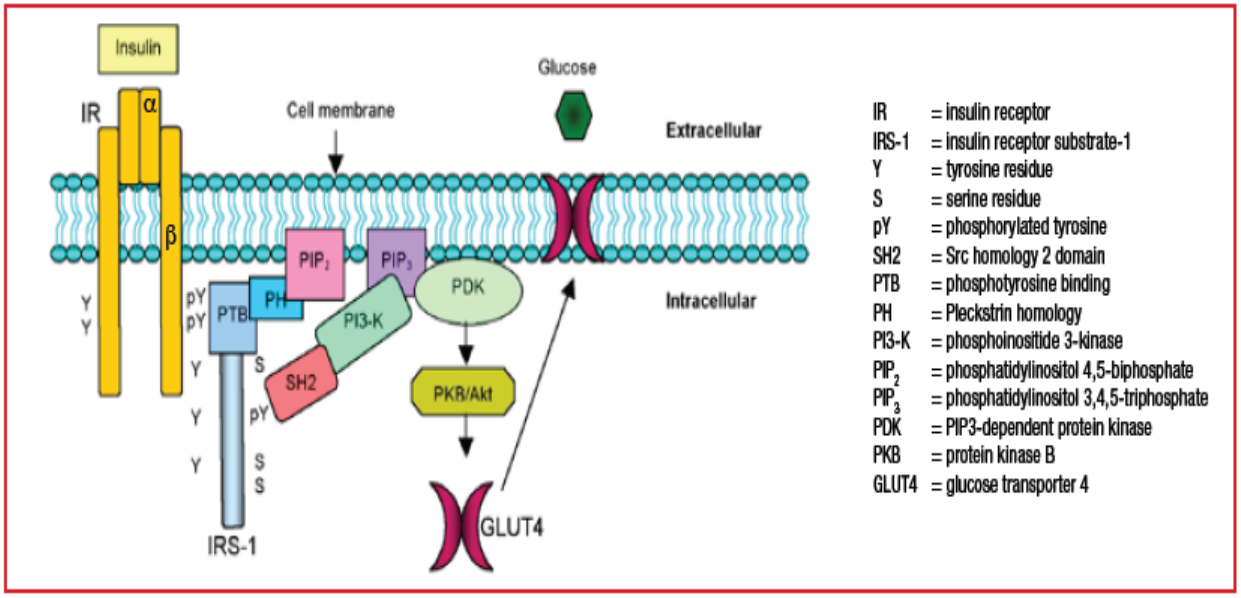

Figure1.4 Insulin signaling pathway

1.1.3. Insulin resistance

On a cellular level, insulin resistance refers to an inadequate strength of insulin signaling from the insulin receptor downstream to the final substrates of insulin action involved in multiple metabolic and mitogenic aspects of cellular function (Ginsberg, 2000).

1.1.4. Dyslipidemia

Dyslipidemia is defined as increased levels of triglyceride, low density lipoprotein cholesterol and total cholesterol and decreased levels of high density lipoprotein cholesterol (Jean, 2006).

1.1.5. Protease inhibitors and insulin resistance

A direct effect of PIs in inducing insulin resistance in patients infected with HIV is suggested by the following: reversal of hyperglycemia after PI withdrawal; onset of hyperinsulinemia before measurable body composition changes in PI recipients; improvement in insulin sensitivity after substitution of the NNRTI nevirapine or the NRTI abacavir for PI; a trend toward reduced insulin sensitivity after only 2 weeks of indinavir monotherapy and an *in vitro* effect of PIs on insulin-stimulated glucose uptake by adipocytes. These all suggest that PIs have a direct effect on inducing insulin resistance in patients infected with HIV (Michael, 2000).

1.1.6. Mechanism of protease inhibitor induced insulin resistance and dyslipidemia

The mechanism involved in the induction of insulin resistance and dyslipidemia by protease inhibitors may be explained by the following observations from the literature: inhibition of the activity of Glucose Transporter 4 in the plasma membrane (Paul et al, 2001; Mustafa et al, 2004 and Vyas et al, 2010), inhibition of preadipocyte/adipocyte differentiation and induction of apoptosis in mature adipocytes, increased VLDL production, impaired triglyceride clearance and mitochondrial dysfunction (Go¨mez et al, 2000; Lagathu and Martine, 2004; Catherine, 2007; Eoin and Patrick, 2011).

1.1.6.1. Protease inhibitors and Glucose Transporter 4 (GLUT 4)

Murata et al (2000) suggested that one of the main mechanisms responsible for the induction of insulin resistance by protease inhibitors is the inhibition of the glucose transporter. As aspartyl protease inhibitors, these compounds all possess a core peptidomimetic structure that resembles the aromatic peptide back bone that serve as the native substrate for the HIV type 1 protease together with flanking hydrophobic moieties. As indicated in figure 1.5, GLUT4 inhibition is produced by direct, noncovalent binding of this core structure of PIs to a unique structural domain within the transport molecule (Johann et al, 2004).

Since the transport of glucose is one of the limiting steps in the elimination of glucose, the inhibitory effect of protease inhibitors on GLUT4 causes insulin resistance in HIV positive individuals that use this class of drugs. Some of these patients may develop diabetes due to the failure of pancreatic β-cells in compensating for this resistance (Andréa et al, 2009).

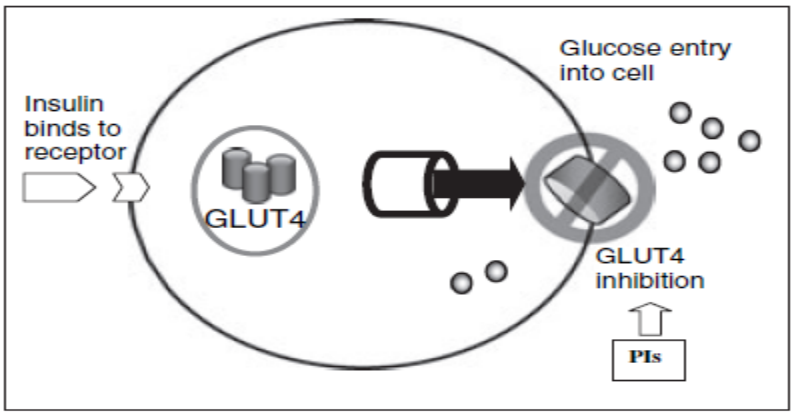

Figure 1.5 Diagrammatic representation of the mode of action of insulin, and mechanism of insulin resistance via GLUT4 inhibition by protease inhibitors (Aboud et al, 2007).

1.1.6.2. Protease inhibitors and adipocytes

The proposed mechanism by which PIs inhibit preadipocyte/adipocyte differentiation and induce apoptosis in mature adipocytes is through their binding to Cytoplasmic Retinoic Acid-Binding Protein type 1 (CRABP-1). CRABP-1 is a ubiquitous protein that binds virtually all intracellular retinoic acid and presents it to cytochrome P450 (CYP) 3A isoforms for conversion to cis-9-retinoic acid, the sole ligand of the retinoid x receptor (RXR) (Figure 1.6). In adipocytes, binding of cis-9-retinoic acid to an RXR activates the receptor, which then forms heterodimers with the gamma subtype of proximal proliferator activated receptor (PPAR□). Binding of the heterodimers to adipocyte retinoic acid response element (RARE) subsequently inhibits apoptosis and stimulates differentiation and proliferation of fat cells (Nageswara et al, 2002; Leonardo et al, 2003and Piliero, 2003).

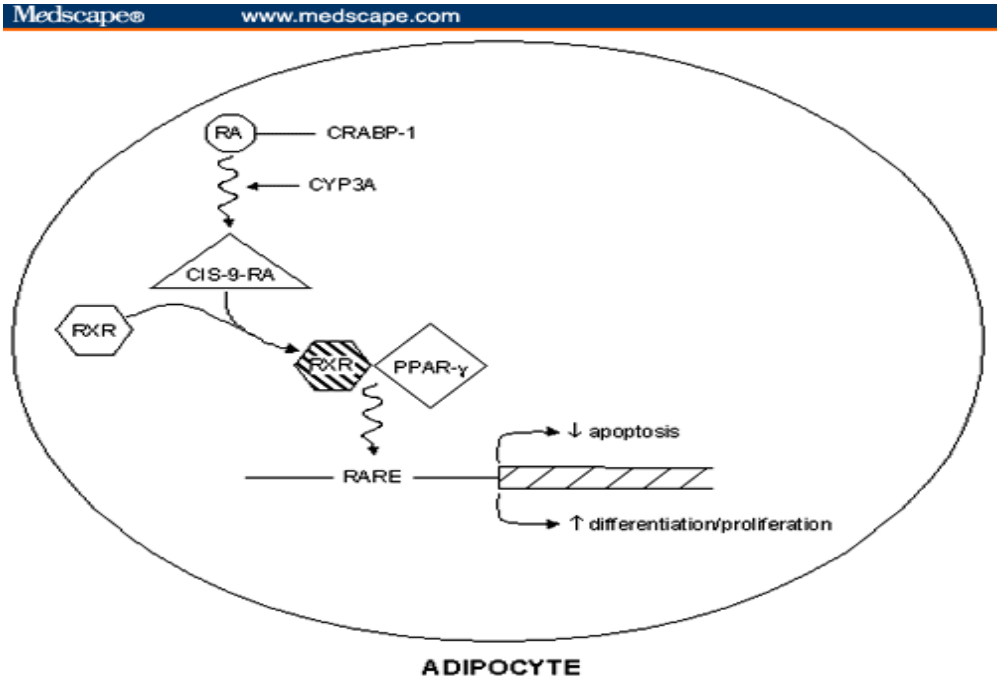

Figure 1.6 the role of CRABP-1 in adipocyte differentiation (Aboud et al, 2007).

PIs have a high affinity for the catalytic site of HIV-1 protease. The 12-amino acid sequence in this HIV-1 catalytic site has a 58% homology with a C-terminal region of CRABP-1. Binding of a PI to these retinoic acid-binding residues of CRABP-1 can inhibit binding of retinoic acid to the transport protein. This inhibition initiates a cascade of events in fat cells, including decreased conversion of retinoids to cis-9-retinoic acid, reduced RXR activation, decreased formation of RXR/PPAR-gamma heterodimers, reduced activation of target genes, reduced adipocyte differentiation and proliferation, and increased adipocyte apoptosis. PIs can also directly inhibit cytochrome P450 3A isoforms that metabolize retinoic acid to cis-9-retinoic acid (cis-9-RA) and lead to the above conditions. These alterations in the number and size of fat cells influence adipose tissue mass and distribution, with resultant changes in carbohydrate and lipid metabolism mainly reduced triglyceride storage and increased lipid release into the circulation, producing hyperlipidemia (Nageswara et al, 2002; Leonardo et al, 2003and Piliero, 2003).

Another mechanism by which protease inhibitors could dysregulate lipid metabolism is by binding to LDL-receptor-related protein (LRP), which has also a 60% sequence homology with the catalytic region of HIV-1 protease , causing impaired chylomicron uptake and triglyceride

clearance by the endothelial LRP-LPL (lipoprotein lipase) complex (Nageswara et al, 2002; Leonardo et al, 2003and Piliero, 2003).

It has also been recently reported that PIs inhibit proteasomal degradation of apolipoprotein B and increase the secretion of apolipoprotein B-lipoproteins. Moreover PI treatment may cause endothelial cell toxicity by damaging mitochondrial DNA (mtDNA) via generation of reactive oxygen species (ROS) leading to impaired β-oxidation followed by hyperlipidemia and vascular complications. PIs also can indirectly affect lipid metabolism by the generation of tumor necrosis factor-α (TNF-α). Dysregulation of TNF-α homeostasis could affect mitochondrial function and alter the lipid metabolism by inhibiting the activity of lipoprotein lipase (Nageswara et al, 2002).

1.2 Review on protease inhibitor induced insulin resistance, glucose metabolism disorder and dyslipidemia

Clinical reports revealed glucose intolerance and insulin resistance in HIV-positive patients treated with PIs (Ismail, 2009). Moreover, the incidence of insulin resistance in HIV-positive patients is significantly higher in PI-treated patients than in patients treated with NRTIs or NNRTIs, even when one considers related factors such as demography and virology. Furthermore, PIs have been shown to induce insulin resistance in HIV seronegative patients and in animal models; implying a clear link between PI use and insulin resistance (Ismail, 2009).

Studies conducted among patients with HIV/AIDS who are receiving non-PI-containing regimens revealed an insulin resistance prevalence rate of about 20% whereas rates as high as 60% to 85% was seen in those receiving a PI-based regimen (Babafemi, 2005). Data from similar studies also suggest that patients receiving therapy with PIs have a greater incidence of insulin resistance (~30%–90%) than patients receiving non-PI-containing regimens (up to 20%) (Marie, 2003).

The observation from longitudinal studies that insulin resistance often precedes lipodystrophy has also suggested that insulin resistance may be the proximate cause of the metabolic syndrome. This is supported by a recent report in which oral glucose tolerance tests and euglycemic clamps were performed on healthy volunteers taking indinavir. Insulin resistance was observed as soon as 4 weeks after start of therapy, before the development of any discernible changes in body fat composition or distribution. Because the volunteers were HIV negative and were not receiving nucleoside analogs, that study suggests that the insulin resistance is a direct effect of PIs (Paul et al, 2001).

In laboratory studies using human 3T3-L1 adipocytes (a type of fat cell), Murata, et al (2000) found that indinavir and other PIs reduced glucose uptake by inhibiting GLUT-4 activity. At 100 micro molar (μm) dose, indinavir reduced glucose uptake by 63% while a 10 μm dose (closer to the concentrations used in humans) caused a 26% decrease. This inhibition occurred within minutes, and was reversed when indinavir was removed (Jean, 2004 and Murata et al, 2002).

In another study in frog egg cells, indinavir, amprenavir, and ritonavir (Norvir) reduced glucose uptake by 45%, 42%, and 54%, respectively. Noting that mutant mice lacking GLUT-4 have almost no subcutaneous (under the skin) fat, the authors suggested that peripheral lipoatrophy (fat loss in the limbs and face) in people with HIV may be mediated by PIs' effect on this transport protein (Murata et al, 2000).

Cross-sectional studies have also documented a high prevalence of insulin resistance and Impaired Glucose Tolerance (IGT) among PI recipients (Behrens et al, 1999). Walli et al performed iv insulin tolerance tests in 67 recipients of nucleoside reverse transcriptase inhibitors (NRTIs) plus PIs (mean duration of treatment, 14 months), 13 therapy-naive subjects, and 18 HIV-negative control subjects. The median level of insulin sensitivity was markedly lower among PI-treated subjects (75 µmol/L/min) than among either control subjects (177 µmol/L/min) or therapy-naive subjects (156 µmol/L/min; P = .001 for the difference between PI-treated and therapy-naive persons). Using a cut-off of 2 SD below the mean for insulin sensitivity among the HIV-negative control subjects, 41 (61%) of 67 PI-treated subjects were considered to have pathological insulin sensitivity, whereas none of 13 therapy-naive subjects exhibited insulin insensitivity (Murata et al, 2000).

Similarly, Behrens et al reported that among PI recipients, 18 (46%) of 38 had IGT detected and 5 (13%) of 38 had diabetes detected by oral glucose tolerance testing, whereas among PI-naive subjects (of whom 10 were receiving NRTIs), 4 (24%) of 17 had impaired glucose tolerance (IGT) and none had diabetes detected (Behrens et al, 1999). In line with this Gobel and Walli also reported higher degree of insulin resistance in 55% of people receiving PI containing HAART as compared with 27% of those receiving NRTIs (Gobel and Walli 1999). Thus, it appears that the prevalence of IGT and insulin resistance is high among subjects infected with HIV who are receiving PIs (Dali et al, 2002 and Marie, 2003).

Related prospective studies have also shown the relatively rapid development of insulin resistance after the initiation of PI therapy. Mulligan et al (2000) reported that in 20 PI-treated subjects who were studied for a mean of 3.4 months after initiation of treatment and were evaluated by use of the homeostasis model assessment (HOMA-IR), fasting insulin and glucose

levels and insulin resistance increased significantly (increases of 96%, 11%, and 149%, respectively). By contrast, there were no significant changes in those parameters in a control group treated with dual nucleoside-based regimens that included lamivudine but did not contain a PI (Marie, 2003 and Jean, 2004).

With regard to the effect of PIs on serum level of lipids, studies show that the frequency and pattern of hyperlipidaemia vary with different antiretroviral agents. Study conducted by Carr et al reported an overall dyslipidemia in 67% of patients receiving PI containing therapy as compared with 26% prevalence in PI-naı¨ve patients (Carr et al, 1999). In another longitudinal study done in Finland, Jussi Sutinen (unpublished) reported that treatment with HAART including a PI for a mean of 3.4 months increased serum total cholesterol by 23% and triglyceride concentration by 48%. Moreover, similar studies also revealed between 47-75% of patients receiving PI developed hyperlipidaemia (Friis-Moller et al, 2003).

In another related prospective study Benesic et al followed 38 HIV-positive receiving a LPV/r containing HAART over a median period of 56 weeks and found that an overall 28 of 38(74%) patients showed hyperlipidemia in which 16 of 38 (42%) hypertriglyceridemia, 2 of 38 (7%) hypercholesterolemia and 10 of 38 (26%) combined hyperlipidemia) (Benesic et al, 2003). However, an increase in LDL cholesterol levels of 25% was also reported with lopinavir/ritonavir therapy of HIV-infected patients in a separate study (Grace et al, 2005).

Similarly a cohort study carried out by Leonardo et al, on 212 HIV-positive patients who started a new PI based antiretroviral regimen and followed for 12 months, showed an incidence of hypertriglyceridaemia and hypercholesterolaemia of 38.2% and 25%, respectively (Leonardo et al, 2004). Moreover, the frequency of increased serum triglyceride levels was also proved to be significantly higher in subjects treated with ritonavir or lopinavir/ritonavir.

In Ethiopia according to the 2007 Single Point Estimate, 336,160 people living with HIV/AIDS (PLHIV) were in need of ART in 2009, out of which 20,522 were children below the age of 15 years. The number of AIDS cases ever started on antiretroviral therapy (ART) has grown to 241,759 as of December 2009, among which 176,632 were currently on treatment – coverage of

53% of those in need. Out of all ART clients 58% (102,379) were female, a reflection of the higher number of women living with HIV. In terms of drug combination, the majority of patients are on the 1st line regimen (like Zidovudin-Lamivudine-Nevirapine); while only 1% (1,079) of the patients has switched to a 2nd line regimen which is lopinavir/ritonavir based combined therapy (FDRE-MOH-HAPCO, 2010).

Thus, in our country, protease inhibitors are given as a component of the second line regimen. Despite the aforementioned metabolic abnormalities induced by these drugs being documented in literatures and proved by studies done abroad, there is no published study that shows the magnitude of problems, in particular insulin resistance and dyslipidemia, associated with PI use in Ethiopia. The purpose of this study is, therefore, to assess insulin resistance and dyslipidemia in HIV-1 infected patients on PI based Combined Antiretroviral Therapy.

1.3. Hypothesis

Insulin resistance and dyslipidemia in HIV-1 infected patients on ART is highly associated with PI-based combined therapy.

1.4. Significance of the study

This study will provide early valuable information on the magnitude of insulin resistance, dyslipidemia and associated impaired carbohydrate metabolism as well as risk of cardiovascular problem in HIV-1 infected patients on PI-based combined antiretroviral therapy. Thereby, the information will enhance awareness of clinicians for the need of close laboratory monitoring, drug switching approach or parallel management of the underlying problems.

2. Objective of the study

2.3. General objective

To assess insulin resistance and dyslipidemia in HIV-1 infected patients on PI based combined antiretroviral therapy.

2.4. Specific objectives

- To compare mean fasting serum glucose values between patients taking PI-based and NNRTI-based Combined antiretroviral therapy
- To determine differences in the mean fasting serum insulin level of HIV-1 infected patients on PI-based combined antiretroviral therapy and NNRTI-based controls
- To determine and compare the percentage of insulin resistance in HIV-1 infected patients on PI-based combined antiretroviral therapy and NNRTI-based controls
- To determine and compare serum lipid profile (total cholesterol, LDL-C, HDL-C and triglyceride) in HIV-1 infected patients on PI-based combined antiretroviral therapy and NNRTI-based controls
- To calculate and compare total cholesterol to HDL ratio between PI-based cases and NNRTI- based controls.
- To calculate and compare triglyceride to HDL ratio between PI-based cases and NNRTI-based controls
- To determine and compare the proportion of patients with metabolic syndrome between PI-based cases and NNRTI-based controls
- To estimate and compare the cardiovascular disease risk among patients on PI-based and NNRTI-based regimen
- To see the relationship between absolute CD+ T cell count and the serum biochemical variables

3. Materials and methods

3.1. Study Design

A cross sectional study with comparative nature was conducted on HIV-1 infected patients receiving PI-based and NNRTI-based combined antiretroviral therapy.

3.2. Study area and subjects

The study was conducted in patients attending ART program at Tikur Anbessa Specialized Teaching Hospital, Addis Ababa, Ethiopia. Tikur Anbessa is the largest tertiary level referral and teaching hospital in the country. Currently 2445 adult patients are on ART follow up from which 2367 are on first line regimen and the remaining 78 are on second line regimen. Study subjects were selected from the total ART attendants based on their availability within the period of study. Those who satisfied the study criteria were informed about the proposed study and their consent was obtained. Investigator administered questionnaire was utilized to gather relevant information from the study participants.

3.3. Population

3.3.1. Source population

All HIV-1 infected patients receiving antiretroviral therapy at Tikur Anbessa Specialized Teaching Hospital.

3.3.2. Study population

HIV-1 infected patients taking PI-based antiretroviral therapy and comparative group who are on NNRTI-based regimen.

3.4. Study variable

3.4.1. Dependent variable

The magnitude of insulin resistance and dyslipidemia in HIV-1 infected patients on PI- based combined antiretroviral therapy.

3.4.2. Independent variables

- Duration of PI-based regimen intake
- Genetic predisposition
- Life style
- Abdominal fat accumulation

3.5. Sample size

This study was conducted on a total of 134 HIV-1 patients which include 67 patients on PI-based regimen and equal number of controls on NNRTI-based regimen. The sample size was determined by considering previous similar comparative German study which reported an insulin resistance of 55% and 27 % on PI-based and PI-naïve regimen respectively (Gobel, 1999) which is also representative for the overall dyslipidemia of 67% in patients on PI-based therapy as compared to 26% in patients on NNRTI-based therapy as reported by Carr et al (1999). The calculation was carried out by the following formula at α =0.05 and 90% power; (Wayne, 2005)

$$n_1 = n_2 = \frac{\left(z_{\alpha/2}\sqrt{2\,\overline{p}\,\overline{q}} + z_{\beta}\sqrt{p_1 q_1 + p_2 q_2}\right)^2}{\Delta^2}$$

Where, $\overline{p} = \frac{p_1 + p_2}{2}$ $\overline{q} = 1 - \overline{p}.$

Δ=p1-p2

$n_1 = n_2$ is the sample size of the study and control group respectively and P_1= 0.55, prevalence of insulin resistance in HIV-1 infected adults on PI based and P_2= 0.27, prevalence of insulin resistance in those on PI- naïve regimen. Thus, $n_1 = n_2$ = 63. According to the formula the minimum sample size was 126 but here 134 subjects were recruited to include almost all the patients who take the PI-based regimen so as to make it more representative.

3.6. Eligibility criteria

Inclusion criteria

- Current recruitment on ART with a minimum of one month intake
- Age ≥18
- Volunteer

Exclusion criteria

Voluntary participants who have one or more of the following conditions that may derange the serum level of glucose, insulin or lipids were excluded from the study.

- Diagnosed diabetes mellitus before initiation of ART
- Diagnosed cardiovascular disease before initiation of ART
- Use of lipid lowering drugs
- Use of either hypoglycemic or hyperglycemic drugs
- Pregnant women
- Lactating mothers

3.7. Ethical consideration

After being approved by the Research and Ethical Committee of the Department of Biochemistry (RECDoB), School of Medicine, College of Health Sciences, Addis Ababa University, formal letter was written to Tikur Anbessa Specialized Teaching Hospital. In addition the whole objective of the study was briefly explained to all concerned authorities as well as technical staff who were assigned in the ART unit so as to get permission and support. Patients who consented to give blood were recruited in the study. Moreover confidentiality was strictly maintained throughout the course of the study and the study outcome was submitted to the hospital as well

3.8. Anthropometric measurements

All anthropometric measurements were made according to the World Health Organization recommendations by paramedical personnel.

Weight and Height

Analog scale with Kg reading was used to measure the weight of study subjects. The subjects were requested to wear minimal clothing and stand on the center of the weight scale platform and recording was taken in kilograms. Height was also measured while they were standing erect there by lowering the horizontal scale bar snugly to the crown of the head. Measurement was recorded to the nearest 0.1 centimeter.

Body mass index (BMI)

Body mass index was calculated from weight (kg) divided by height squared (m^2). According to world health organization (WHO) Participants were classified as underweight (< 18 kg/m^2), normal (18 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2) or obese (> 30 kg/m^2) (WHO, 1998).

Waist circumference

The subjects were requested to wear little clothing and stand with feet close together, arms at the side and body weight evenly distributed. More over they were asked to relax and measurement was taken at the end of normal exhalation at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest, using a stretch-resistant tape that was held snug around the body. Each measurement was repeated twice and the average of measurements that are within 1 cm of one another was recorded to the nearest 0.1 cm.

Hip circumference

The subjects were requested to wear little clothing and stand with feet close together, arms at the side and body weight evenly distributed. Hip circumference measurement was taken around the widest portion of the buttocks using a stretch-resistant tape that snug around the body. Each measurement was repeated twice and the average of measurements that are within 1 cm of one another was recorded to the nearest 0.1 cm.

Waist to hip ratio (WHR)

Waist to hip ratio was computed from the already measured waist and hip values using the following formula;

WHR = Waist circumference/Hip circumference. According to WHO guide line, participants were stratified using the cutoff for substantial risk of metabolic complication which is ≥ 0.90 for males and ≥ 0.85 for females (WHO, 2008).

3.9. Blood pressure measurement

Blood pressure of the subjects was also measured according to the WHO recommendation using a sphygmomanometer. The participants were allowed to sit on a chair for at least five minutes and rest their arm palm-up on a table so the brachial artery was level with the heart. Then blood pressure was measured using an inflatable cuff wrapped around the subject's left upper arm, just above the elbow and the stethoscope placed on the hollow of the elbow, that is just below the cuff on the inside of the arm and directly over the brachial artery. Both systolic and diastolic blood pressure measurements were taken in units of millimeter mercury.

3.10. Specimen collection and handling

About 5 ml of venous blood was collected aseptically from overnight fasting patients by a vaccutainer system on SST^{TM} test tube. Specimen was allowed to clot for 30 minutes. The clot was gently dislodged using an applicator stick and the serum separated by centrifuging at 3500 rpm for 5 minutes. Then after serum samples were aliquoted in to nunc tubes aseptically by using a micropipette with disposable sterile blue tips. Finally serum glucose was analyzed immediately and the aliquots were stored at -70^{o}c until the time of analysis.

3.11. Quality assurance

To maintain the final quality, specimens were collected in an aseptic technique, processed properly and analyzed carefully on calibrated clinical chemistry analyzers in parallel with controls following standard operating procedures. Finally results were interpreted in reference to normal ranges and then followed by appropriate statistical analysis.

3.12. Methods of Biochemical analysis

Fasting Serum samples were analyzed for glucose, insulin, total cholesterol, LDL-C, HDL-C and triglyceride. Level of insulin resistance was calculated using HOMA-IR from glucose and insulin. The atherogenic indices total Cholesterol to HDL-C and triglyceride to HDL-C ratios were also computed from the serum lipids. The analysis was carried at Tikur Anbessa Specialized Teaching Hospital and Ethiopian Health and Nutrition Research Institute (EHNRI) clinical chemistry laboratories using Humalyzer 300 for glucose, Rochi Elecsys 2010 for insulin and Cobas Integra 400 plus for the lipid profile.

3.12.1. Determination of glucose

Glucose oxidase method

PRINCIPLE: Glucose oxidase (GOD) catalyses the oxidation of β-D-glucose to gluconic acid with the concurrent release of hydrogen peroxide. The formed hydrogen peroxide (H_2O_2) is detected by a chromogenic oxygen acceptor, phenol-aminophenanzone in the presence of peroxidase (POD):

(1) β-D-Glucose + O_2 + H_2O ----- GOD ----->Gluconic acid + H_2O_2

(2) H_2O_2 + phenol+ aminophenanzone --(POD) --------> quinone + H_2O

The absorbance of quinone is measured at 505 nm and is proportional to the concentration of glucose in the sample. (Human, Germany)

Table 3.1 Content of glucose assay kit

Reagent 1 (R1) Buffer	TRIS pH 7.4----------92mmol/L Phenol ---------- 0.3 mmol/L
Reagent 2 (R2) Enzymes	Glucose oxidase (GOD)----------15000 U/L Peroxidase (POD) ---------- 1000 U/L 4- Aminophenonzone (4 - AP) ---2.6 mmol/L
Glucose CAL	Glucose aqueous primary standard --- 100mg/dl

Working reagent (WR): Working reagent was prepared by dissolving the contents of one vial R2 enzymes in one bottle of R1 buffer which then caped and mixed gently to dissolve the contents. The reagent is stable 1 month after reconstitution in the refrigerator (2-8 ^{0}c) or 7 days at room temperature (15-25 ^{0}c).

Procedure

1. Sample, standard and reagent were brought to room temperature.
2. Three plain tubes were prepared and label as blank, standard and test.
3. The assay mixture was prepared in each tube as follows:

Table 3.2 Glucose assay mixture

	Blank	Standard	Sample
WR (ml)	1.0	1.0	1.0
Standard (µL)	--	10	--
Sample (µL)	--	--	10

4. The assay mixture in each tube was Mixed and incubated for 10 min at 37 ^{0}c.
5. Finally the absorbance (A) of the sample and standard was read against the reagent blank.

Calculation

Glucose concentration can be obtained by $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ X 100 (standard conc.)

= mg/dl glucose in the sample but the spectrophotometer computes by itself and automatically result was displayed in mg/dl.

3.12.2. Determination of insulin

Insulin immunoassay

Principle: -The Elecsys electrochemiluminesence insulin assay which is based on the sandwich immunoassay approach employs two monoclonal antibodies, biotnylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody labeled with ruthenium complex[a], which together are specific for human insulin and a substrate streptavidin-coated microparticles. The two monoclonal antibodies form a sandwich complex with serum insulin in the first

incubation followed by the binding of streptavidin to the biotnylated monoclonal insulin-specific antibody in the second incubation. Finally the reaction mixture is aspirated in to the measuring cell were the voltage induced chemiluminescence is measured by a photomultiplier tube.

Procedure

- 1st incubation: 20 µl of sample was mixed with a biotnylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody labeled with ruthenium complex[a] to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex became bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture was then aspirated in to the measuring cell where the microparticles were magnetically captured on to the surface of the electrode. Unbound substances were removed from the proCell. Application of a voltage in to the electrode then induced chemiluminescent emission, which was measured by a photomultiplier tube. Results were determined via a calibration curve generated by 2-point calibration and a master curve provided via the reagent barcode.(Roche diagnostics GmbH, D-68298 Mannheim, Germany)

  Reagents- Working solutions

  M Streptavidin – coated microparticles (transparent cap), 1 bottle, 6.5ml

  R1 Anti-insulin-Ab~biotin (gray cap), 1bottle, 10ml

  R2 Ant-insulin-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10ml

  Calibrators

  - Insulin cal1: 2 bottles, each for I.0 ml of calibrator 1
  - Insulin cal2: 2 bottles, each for 1.0 ml of calibrator 2

3.12.3. Estimation of insulin resistance

Homeostasis model assessment (HOMA) method

HOMA was first developed in 1985 by Matthews et al (Matthews, 1985). It is a method used to quantify insulin resistance by means of a set of simple, mathematically-derived equation derived from the use of the fasting serum insulin glucose product divided by a constant, **HOMA-IR =**

[glucose (mmol/L) × insulin (µU/L)]/22.5 (Singh and Saxena , 2010). It is an alternative to the gold standard hyperinsulinemic euglycemic glucose clamp and the most commonly used surrogate measure of insulin resistance in vivo. In terms of precision (reproducibility of measure), HOMA-IR is comparable to the clamp technique. HOMA-IR is inferior to the clamp technique in terms of accuracy, but using HOMA-IR makes it possible to study a large number of subjects with a single glucose and insulin measurement in the fasting state (Bonora et al, 2000).

3.12.4. Determination of triglyceride

Principle: Triglycerides are hydrolyzed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to glycerol-3-phosphate (G-3-P), which is oxidized by the enzyme glycerol-3-phosphate oxidase (GPO) producing hydrogen peroxide. Hydrogen peroxide, so formed, reacts with 4-aminoantipyrine and 4-chlorophenol in the presence of enzyme POD to produce Quinoneimine dye with maximum absorption at 538nm**.** (Roche diagnosticsGmbH, D-68298 Mannheim, Germany)

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol + Fatty Acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2$$

$$H_2O_2 + \text{4-AA + 4-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoeimine Dye+ HCL + } 2H_2O$$

Procedure

1. Sample, calibrator, control and reagents were brought to room temperature.
2. Then 500 µl each of sample, control and calibrator was measured and dispensed in to sample cup.
3. The reagent and the measured solutions were positioned in to their respective locations in the Cobas Integra 400 plus automated analyzer.
4. Finally the reaction mixture was prepared as in table 3.3, incubated and absorbance read at 538 nm with an automatic display of triglyceride concentration in mg/dl.

Table 3.3 Triglyceride assay mixture

	Volume (µl)	Diluent (H_20)
Working reagent	120	
sample	2	28
Total volume	150	

3.12.5. Measurement of total cholesterol

Principle: Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase (CE). The free cholesterol produced is oxidized by cholesterol oxidase (CO) to cholesten-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to yield Quinoneimine dye with maximum absorption at 538 nm (Roche diagnostics GmbH, D-68298 Mannheim, Germany).

Cholesterol ester + H_20 $\xrightarrow{Cholesterol}$ Cholesterol + fatty acids

Cholesterol + O_2 $\xrightarrow{Cholesterol\ oxidase}$ 4-Cholestinone + H_2O_2

H_2O_2 + phenol + 4-aminoantipyrine $\xrightarrow{Peroxidase}$ Quinoneimine dye + H_2O

.

Procedure

1. Sample, calibrator, control and reagents were brought to room temperature.
2. Then 500 µl each of sample, control and calibrator was measured and dispensed in to sample cup.
3. The reagent and the measured solutions were positioned in to their respective locations in the Cobas Integra 400 plus automated analyzer.
4. Finally the reaction mixture was prepared as in table 3.4, incubated and absorbance read at 538 nm with an automatic display of total cholesterol concentration in mg/dl.

Table 3.4 Total cholesterol assay mixture

	Volume (µl)	Diluent (H_20), µl
Working reagent	120	
sample	2	28
Total volume	150	

3.12.6. LDL measurement

Hemogenous (direct) enzymatic colorimetric assay

Principle: The LDL-Cholesterol plus 2nd generation test principle is based on the selective micellary solubilization of HDL-Cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins. The inclusion of a sugar compound and a detergent in the enzymatic method for cholesterol determination (cholesterol esterase - cholesterol oxidase coupling reaction) enables the selective determination of LDL-cholesterol. The color intensity of the blue quinoneimine dye formed is directly proportional to the LDL-cholesterol concentration. It is determined by measuring the increase in absorbance at 583 nm.

LDL-cholesterol ester + H_20 $\xrightarrow{\text{detergent cholesterol esterase}}$ Cholesterol + free fatty acid
(selective micellary solubilization)

LDL-cholesterol + 0_2 $\xrightarrow{\text{cholesterol oxidase}}$ Δ4-cholestenone + H_20_2

$2H_20_2$ + 4-aminoantipyrine + HSDA + H^+ + H20 $\xrightarrow{\text{peroxidase}}$ purple blue pigment + $5H_20$

Reagents-Working solution

R1 Buffer in vial A (liquid) and in vial B (liquid)

R2= SR enzymes in vial c (liquid)

Procedure

1. Sample, calibrator, control and reagents were brought to room temperature.
2. Then 500 µl each of sample, control and calibrator was measured and put in to sample cup.
3. The reagent and the measured solutions were positioned in to their respective locations in the Cobas Integra 400 plus automated analyzer.

4. Finally the reaction mixture was prepared as in table 3.4, incubated and absorbance read at 538 nm with an automatic display of LDL cholesterol concentration in mg/dl.

Table 3.5 LDL- Cholesterol assay mixture

	Volume (µl)	Diluent (H_20), µl
Working reagent	150	
sample	2	7
SR	50	
Total volume	209	

3.12.7. HDL measurement

Precipitation method

Principle: The very low density (VLDL) and low density (LDL) lipoproteins from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After removed by centrifugation the clear supernatant containing high density lipoproteins (HDL) is used for the determination of HDL using the total cholesterol reagent.

Precipitation procedure

1. A centrifuge tube was labeled as HDL and 100 µl precipitating reagent was mixed with 1000 µl of serum.
2. The Mixture was mixed well and allowed to stand for 10 minutes at room temperature
3. At the end of incubation time the mixture was centrifuged at 4000 rpm for 20 minutes.
4. Finally the supernatant was Collected and tested for HDL-C using the total cholesterol reagent.

3.12.8. Total cholesterol to HDL ratio

Total cholesterol to HDL ratio was calculated by dividing total cholesterol to HDL value and value greater than 5 was taken as indicator of average risk to cardiovascular disease according to the National Cholesterol Education Program ATP III.

3.12.9. Triglyceride to HDL ratio

Triglyceride to HDL ratio was calculated by dividing serum triglyceride to serum HDL and value greater than 3.8 was considered as indicative of cardiovascular disease risk (Protasio et al, 2008).

3.13. Data Analysis

Data was entered into excel sheet, cleaned and analyzed using SPSS version 16. Kolmogorov-Smirnova and visual inspection of histogram was utilized to test for Gaussian distribution of the data and log and inverse transformation were applied where appropriate. Descriptive analysis was done to assess association between regimen type (PI, NNRTI-based) and insulin resistance and dyslipidemia. Independent t-test, Chi-square test (χ^2) results and 95% confidence interval was used to measure the strength of association. The non-parametric Mann-Whitney u was also used to compare mean values of continuous variable with non-normally distributed data. Furthermore, Pearson's correlation was used to see the relationship between the continuous variables. P values less than 0.05 was considered as significant for all.

4. Results

4.12. Demographic characteristics of the study participants

A total of 134 HIV-1 positive patients on ART, 67 taking PI-based regimen as a case group and 67 taking NNRTI-based regimen as a control group were recruited to participate in this study. All of them were voluntary to respond to the investigator-administered questionnaire, give the required volume of blood and signed the consent form. As represented in Table 4.1 the percentage of males and females was 49.3% and 50.7% in the PI-based and 41.8% and 58.2% in the NNRTI-based controls. The age of the study participants ranges from 20 - 68 years and the mean age was 40.9 and 42.2 in the cases and controls respectively.

4.13. Questionnaire variable summaries

All the subjects in this study were HIV-1 patients on ART either on the PI-based (cases) or NNRTI-based (controls). The duration of ART, type of regimen and drug combination was collected from their medication chart. The duration of ART intake in those currently on PI-based regimen ranges from 6-94 overall (2-68 on PI-based) in months while in those on NNRTI-based regimen ranges from 3 – 101 months. The median duration was found to be 60 overall (27on PI-based) among the PI-based group as compared to 51 months in the NNRTI-based regimen. Overall 19 (14.2%) of the patients reported that they had family history of diabetes mellitus. 12/67 (17.9%) were in the PI-based regimen while 7/67 (10.4%) were in the NNRTI-based regimen. Almost all except 2 (1.4%) patients involve in activities that need moderate exercise and only one mother had reported that she had gestational diabetes as shown in Table 4.1. The medication charts also revealed that most of the patients use three antiretroviral drugs in combination; one from either of the NNRTIs (nevirapine or efaverinz) or protease inhibitor lopinavir/ritonavir in combination with two drugs from either of the NRTIs as summarized in Table 4.2. Majority of the cases take TDF/ 3TC/ LPV/r (61.2%) followed by AZT/3TC/LPV/r (19.4%) and ABC/3TC/LPV/r (6%) while the controls take TDF/3TC/NVP (55.2%) followed by AZT/ 3TC/ EFV (22.4%) and AZT/ 3TC/ NVP (14.9%) respectively.

Table 4.1 Summary of base line characteristics of the study subjects

Variable	PI-based	NNRTI-based	P. value
Sex, n (%)			
Male	33(49.3)	28 (41.8)	NA
Female	34 (50.7)	39 (58.2)	NA
Age (years)			
Median (1^{st} - 3^{rd} quartile)	40 (34 - 49)	41(35 - 48)	0.45
Range	23 – 69	20 - 62	
Duration of ART (months)			
Median (1^{st} - 3^{rd} quartile)	60 (35 -71)	51(28 - 64)	0.002
Range	6 - 94	3 – 101	
Absolute CD4+ T cell count (cells/mm^3)			
Median (1^{st} - 3^{rd} quartile)	304(208 - 424)	301(244 - 449)	0.35
Range	22 – 742	76 – 804	
Family history of Diabetes Mellitus, n (%)	12 (17.9)	7(10.4)	NA
Gestational diabetes, n (%)	0	1/39(2.7)	NA
Cigarette smokers, n (%)	1(1.5)	0	NA

NA – not applicable

Table 4.2 Frequency and combination of drugs used by patients on PI-based and NNRTI-based regimen

Regimen category	Drug combination	Number of patients	Percentage
PI-based	**TDF/ 3TC/ LPV/r**	**41**	**61.2**
	AZT/3TC/ LPV/r	**13**	**19.4**
	ABC/ 3TC/ LPV/r	**4**	**6.0**
	TDF/ddI/ LPV/r	3	4.5
	ABC/ddI/ LPV/r	2	3.0
	ABC/ddI(EC)3TC/LPV/r	1	1.5
	ddI(EC)/ 3TC/ LPV/r	1	1.5
	TDF/ ABC/ LPV/r	1	1.5
	TDF/CBV/LPV/r	1	1.5
	Total	67	100.0
Non-PI based	**TDF/ 3TC/ EFV**	**37**	**55.2**
	AZT/ 3TC/ EFV	**15**	**22.4**
	AZT/ 3TC/ NVP	**10**	**14.9**
	TDF/ 3TC/ NVP	4	6.0
	D4T/ 3TC/ NVP	1	1.5
	Total	67	100.0

Anthropometric measurements

Body mass index (BMI)

As shown in Table 4.3, the 5% trimmed mean value of BMI among PI-based was 22.1 +/- 3.23 kg/m^2 which was greater than that observed among the non-PI-based, 20.87 +/- 2.46 kg/m^2. The difference was statistically significant at p = 0.021, 95% CI (0.189, 2.230). Moreover, a greater proportion of individuals in the PI-based, 19/67 (28.4%) were found to be overweight as compared to the NNRTI- based, 7/67 (10.4%). The difference was highly statistically significant, $X^2 = 6.872$, df = 1, P = 0.009 (Table 4.4).

Waist to hip ratio (WHR)

The 5% trimmed mean value of WHR in the PI-based was found to be 0.86 +/- 0.52 which was greater than 0.85 +/- 0.52 for the NNRTI- based (Table 4.3). The mean difference between the two groups was statistically significant, p = 0.048 95% CI (0.001, 0.1369) even though not clinically relevant.

4.14. Blood pressure measurement

Systolic blood pressure

The 5% trimmed mean systolic blood pressure in the PI-based was 110 +/- 12.26 mmHg, which was similar with that of NNRTI-based group, 110 +/- 11.9 mmHg (Table 4.3). There was no statistically significant mean difference between the cases and controls, P = 0.796, 95% CI (-3.734, 4.860). The percentage of individuals with systolic blood pressure above the cut off (>120mmHg) among the PI-based was found to be higher than those in the NNRTI-based, 11/67 (16.4%) and 6/67 (9%), respectively, but the difference was not statistically significant, X^2= 1.684, df = 1, P = 0.194 (Table 4.4).

Diastolic blood pressure

The 5% trimmed mean value of diastolic blood pressure for the PI-based was 74+/- 9.14 mmHg, which was almost the same as of the NNRTI-based, 75 +/- 10.26 mmHg (Table 4.3). Again the mean difference was not statistically significant, p = 0.678, 95% CI (-4.180, 2.728). The proportion of subjects who have a diastolic blood pressure above the cut off (>80 mmHg) in the

PI-based was 11 (16.4%) as compared to 10 (14.9%) in the NNRTI-based. The variation was not statistically significant, $X^2 = 0.056$, df = 1, p = 0.812 (Table 4.4).

Table 4.3 Comparison of anthropometric and blood pressure measurement means between patients taking PI-based and NNRTI-regimen

Vatriable	Mean ± SD in PI-based	Mean ± SD in NNRTI-based	Mean difference	(95% CI)	p. value
WHR	0.86 +/- 0.52	0.85 +/- 0.52	0.019	(0.0001, 0.0369)	**0.048***
BMI(Kg/m^2)	22.1 +/- 3.23	20.9 +/- 2.5	1.21	(0.189, 2.230)	**0.021***
SBP(mmHg)	110 +/- 12.3	110 +/- 11.9	0.56	(3.734, 4.860)	0.796
DBP(mmHg)	74 +/- 9.14	75 +/- 10.3	-0.73	(-4.180, 2.728)	0.678

Data presented as mean ± standard deviation, WHR-waist to height ratio, BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure*p<0.05

Table 4.4 Comparison of the proportion of subjects with anthropometric and blood pressure measurements above cut off value between patients taking PI-based and non-PI based regimen

Variable		Cut off	N(%) patients on PI-based above cut off	N (%) patients on NNRTI- based above cut off	X^2	p. value
WHR	M	≥0.90	6/33 (18.2%)	10/28 (35.7%)	1.585	0.21
	F	≥0.85	17/34 (50%)	17/39 (43.6%)	0.300	0.584
BMI		≥25 Kg/m^2	19/67 (28.4%)	6/67 (10%)	6.872	**0.009****
Systolic BP		>120 mmHg	11/67 (16.4%)	6/67 (9%)	1.684	0.194
Diastolic BP		>80 mmHg	11/67 (16.4%)	10/67 (14.9%)	0.056	0.812

WHR-waist to height ratio, BMI-body mass index, **p<0.01

4.15. Biochemical variables

4.15.8. Parameters for assessing insulin resistance

Glucose

The 5% trimmed mean value of fasting serum glucose level in the PI-based was 5.1 +/- 0.85 mmol/L and that of the NNRTI-based was 5.33 +/- 0.79 mmol/L. The mean difference was not statistically significant, p =0.061, 95% CI (-0.5736, -0.013) (Table 4.5). The proportion of individuals who developed hyperglycemia among the PI-based was 14.9% as compared to 19.4% in the NNRTI-based. The proportion however was not statistically significant, $X^2 = 2.001$,df = 1, p= 0.369.

Insulin

The 5% trimmed mean value of fasting serum insulin level in the PI-based was 10.14 +/- 5.8µU/L which is higher than the mean value observed in the NNRTI-based, 8.7 +/- 5.3 µU/L. But there was no statistically significant mean difference between the two groups, p=0.149, 95% CI (-0.5259, 3.4234) (Table 4.5). The proportion of individuals who developed hyperinsulinemia among the PI-based was 3% which was somewhat lower than that obtained for the NNRTI-based, 7.5%. But the difference was not statistically significant, X^2=1.35, df = 1, p=0.244.

Homeostatic model assessment of insulin resistance (HOMA-IR)

The 5% trimmed mean value of HOMA-IR in the PI-based was 2.4 +/- 1.75, which is somewhat higher than that of the NNRTI-based group, 2.2 +/- 1.68. The difference between the two means was not statistically significant, p=0.542, 95% CI (-0.4221, 0.7998) (Table 4.5). The prevalence of insulin resistance among the PI-based was 34.3% which was higher than that observed for the NNRTI-based controls, 28.4% (Figure 4.1). The difference in the proportions however was not statistically significant, $X^2 = 0.555$, df = 1, p=0.456.

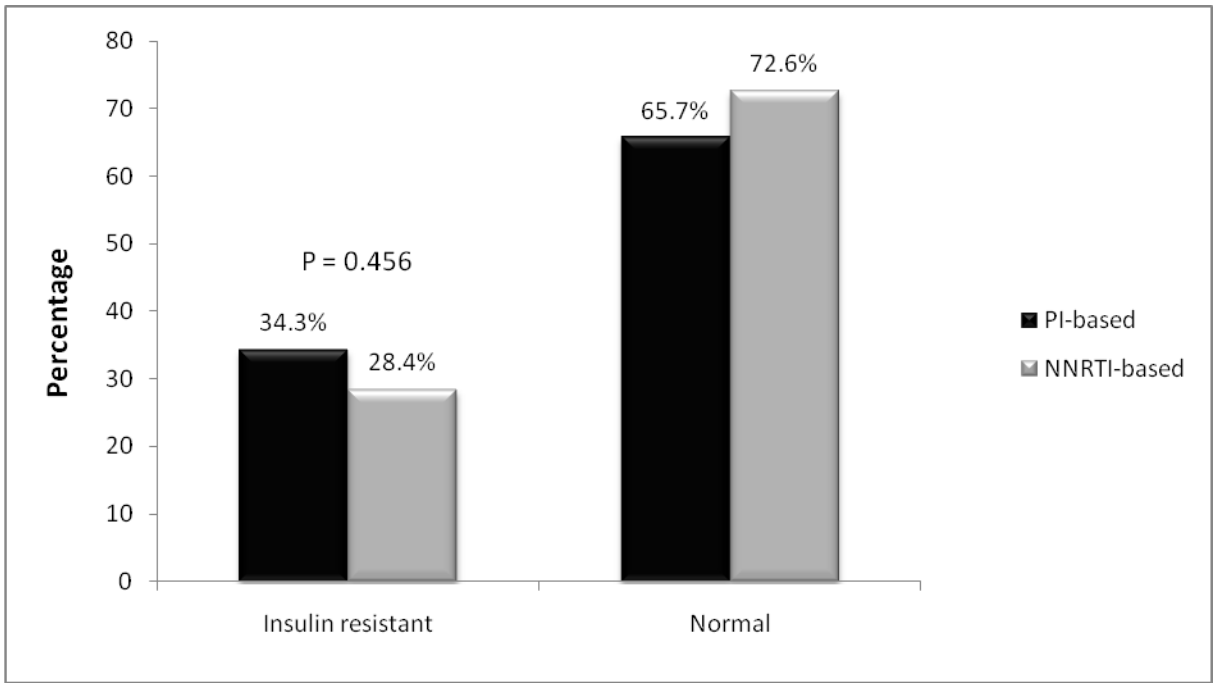

Figure 4.1 Prevalence of insulin resistance among patients taking PI-based and NNRTI-based regimen (n = 67). HOMA-IR cut off >2.5

4.15.9. Parameters for assessing dyslipidemia

Triglyceride

The 5% trimmed mean value of serum triglyceride level among the PI-based was 2.7 +/- 1.5 mmol/L as compared to 1.9 +/- 1.4 mmol/L in the NNRTI- based. The mean difference observed was highly statistically significant P=0.003, 95% CI (0.25, 1.3) (Table 4.5). Moreover, according to the National Cholesterol Education Program Adult Treatment Panel III (2000) criteria, the percentage of individuals who developed hypertriglyceridemia (>1.7 mmol/L) among the PI-based, 74.6% was much higher than that observed in the NNRTI-based controls, 34.3% (Figure 4.2). Thus, the prevalence of hypertriglyceridemia among those who take PI-based regimen was more than twice than that revealed in those who take NNRTI-based regimen, The variation in the proportions was also highly statistically significant, X^2= 21.937,df = 1, p < 0.001.

Total cholesterol

The 5% trimmed mean value of total cholesterol in the PI-based cases was 5.4 +/- 1.1 mmol/L which was slightly higher as compared to the mean value for the NNRTI-based group, 5.1 +/- 0.9 mmol/L (Table 4.5). However the observed variation between the two means was not statistically significant p= 0.137, 95% CI (-0.08, 0.6). According to NCEP ATP III (200) criteria, the percentage of subjects who developed hypercholesterolemia(>5.1 mmol/L) in those on PI-based regimen, 58.2% was also non-significantly higher than 47.8% that observed in the NNRTI- based controls, X^2= 1.468, df =1, p=0.229 (Figure 4.2).

Low density lipoprotein cholesterol (LDL-C)

The 5% trimmed mean value of LDL cholesterol in the PI-based was 2.8 +/- 0.8 mmol/L which was almost similar with the mean of the NNRTI-based controls, 2.9 +/- 0.8 mmol/L (Table 4.5). Thus the variation was not statistically significant, p = 0.564, 95% CI (-0.46, 0.25). Moreover, based on NCEP ATP III (2002) criteria, an equally higher proportion of subjects, 65.7% and 64.2% in the PI-based and NNRTI-based group respectively had been found to have elevated LDL cholesterol (> 2.6 mmol/L) (Figure 4.2). The variation observed between the regimens was not statistically significant, X^2=0.033, df =1, p=0.856.

High density lipoprotein cholesterol (HDL-C)

The 5% trimmed mean value of HDL in the PI-based group was 1 +/- 0.3 mmol/L which was smaller than that of 1.1 +/- 0.28 mmol/L for the NNRTI-based controls. The mean difference between the two regimens was not statistically significant, p = 0.201, 95 % CI (-0.17, 0.04) (Table 4.5). Based on NCEP ATP III (2000) criteria, the proportion of males who had low HDL level (<1.03 mmol/L) among the PI based and NNRTI based was 60.6% and 46.4% respectively (Figure 4.2). The variation was not statistically significant, X^2 = 1.226, df =1, p= 0.27. Likewise the proportion of females with lower HDL value (<50mg/dl) was found to be 82.4% and 82.1 % among the PI-based and NNRTI- based respectively (Figure 4.2). Again the variation in proportion was not statistically significant, X^2 = 0.001, df = 1, P = 0.97.

Total cholesterol to HDL ratio

The 5% trimmed mean value of calculated total cholesterol to HDL ratio for the PI-based 5.7+/- 1.7 was greater than that obtained for the NNRTI-based controls, 5.0 +/- 1.4 (Table 4.5). The mean difference between the regimens was statistically significant, p=0.026, 95% CI (0.0819, 1.2148). Moreover the proportion of patients with calculated total cholesterol/HDL above the risk indicator (>5) observed for the PI-based group, 61.2% was by far higher than that of 41.3% observed for the NNRTI-based group. The observed variation was also highly statistically significant, $X^2 = 5.852$, df = 1, p=0.016 (Figure 4.2).

Triglyceride to HDL ratio

The 5% trimmed mean value of calculated triglyceride to HDL ratio obtained for the PI-based group was 6.96+/- 4.6 which was by far greater than 3.98 +/- 2.69 that for the NNRTI-based group (Table 4.5). The mean difference between the regimens was highly statistically significant, p = 0.007, 95% CI (0.5911, 3.7063). Furthermore the proportion of subjects with calculated TG/HDL above the risk indicator (>3.8) among the PI-based was 74.6% which was much higher than the 38.8% observed among the NNRTI-based controls, which also further escalate the risk of cardiovascular disease among the PI-based cases (Figure 4.2). The observed variation in proportion between the cases and the controls was highly statistically significant, $X^2 = 17.51$, df = 1, p<0.001.

Table 4.5 Comparison of the means values of biochemical variables between patients taking PI-based and NNRTI-based regimen

Variable	Mean ± SD in PI- based	Mean ± SD in NNRTI based	Mean difference	95% Confidence interval	p. value
Glucose (mmol/L)	5.1 +/- 0.85	5.33 +/- 0.79	-0.23	(-0.57,-0.013)	0.061
Insulin(µU/L)	10.14 +/- 5.79	8.7 +/- 5.29	1.44	(-0.53,3.42)	0.149
HOMA	2.4 +/- 1.75	2.2 +/- 1.68	0.20	(-0.42, 0.79)	0.542
TG (mmol/L)	2.7 +/- 1.5	1.9 +/- 1.4	0.8	(0.25, 1.3)	**0.003****
TC (mmol/L)	5.4 +/- 1.1	5.1 +/- 0.9	0.3	(-0.08, 0.6)	0.137
HDL-C (mmol/L)	1 +/- 0.3	1.1 +/- 0.28	-0.1	(-0.17, 0.04)	0.201
LDL-C (mmol/L)	2.8 +/- 0.8	2.9 +/- 0.8	-0.1	(-0.46, 0.25)	0.584
TC /HDL	5.7+/- 1.7	5.0 +/- 1.4	0.7	(0.082, 1.21)	**0.026***
TG/HDL	6.96+/- 4.6	3.98 +/- 2.69	2.98	(1.601, 4.364)	**<0.001****

Data presented as mean ± standard deviation, HOMA - homeostasis model assessment of insulin resistance,TG-triglyceride, TC- total cholesterol, TC/HDL - total cholesterol to high density lipoprotein ratio, TG/HDL - triglyceride to high density lipoprotein ratio**,** * p<0.05, **p<0.01

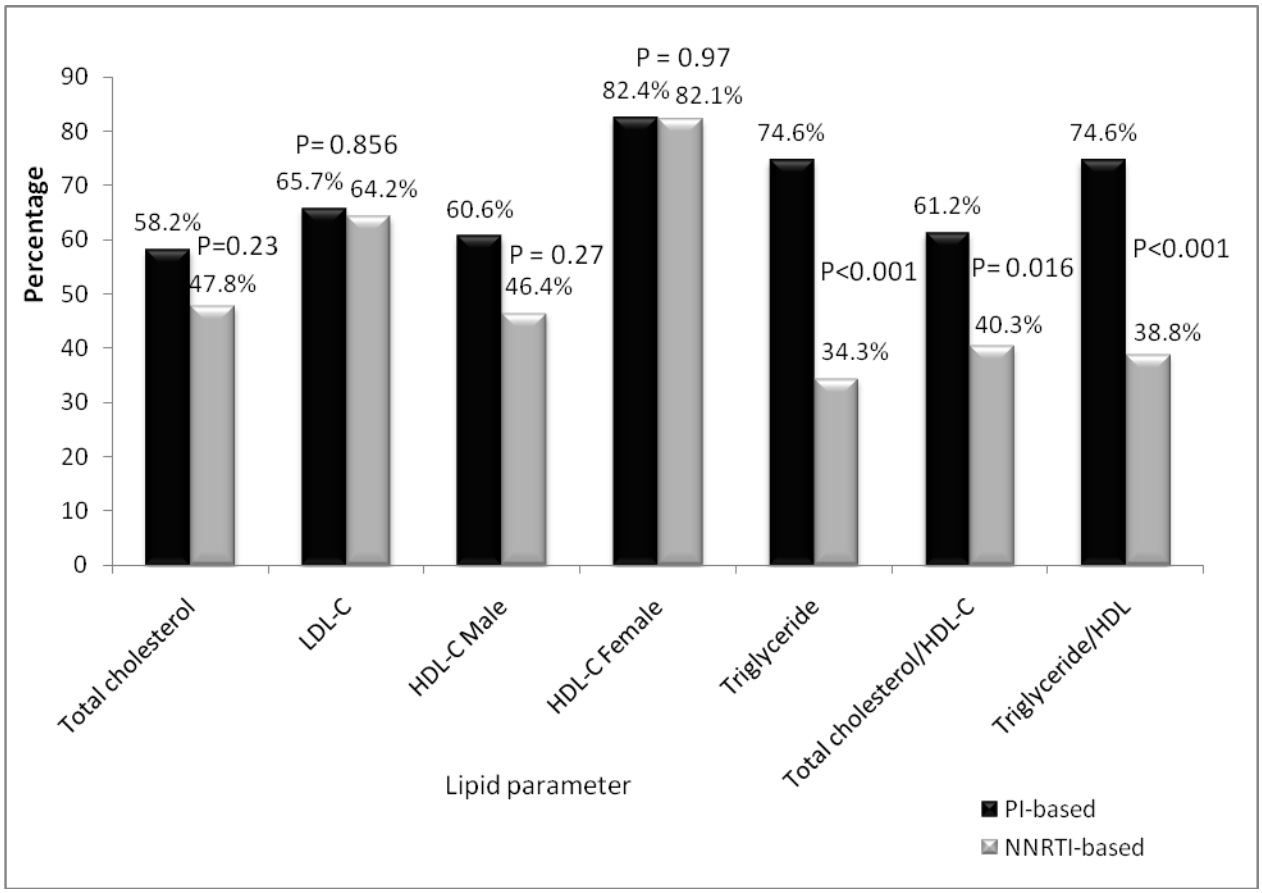

Figure 4.2 Percentage of patients with lipid abnormalities among PI-based and non-PI based regimen (n = 67). [Cut off values: total cholesterol >5.1 mmol/L, LDL-C >2.6 mmol/L, HDL-C <1.03mmol/L for male and <1.28 mmol/L for female, triglyceride >1.7 mmol/L, total cholesterol to HDL C ratio >5 and triglyceride to HDL-C ratio >3.8] according to NCEP ATP-III, 2002 and Protasio et al, 2008.

Prevalence of Metabolic syndrome

Metabolic syndrome (MetS) encompasses a cluster of coronary heart disease and diabetes mellitus risk factors, including abdominal obesity, glucose intolerance, dyslipidemia and elevated blood pressure. Presently there are a couple of sets of defining criteria for metabolic syndrome. According to American Heart Association/Updated NCEP, an individual is diagnosed as having metabolic syndrome if either three of the following coexist: elevated waist circumference >102 for men and > 88 cm for women, Plasma triglycerides 1.7 mmol/L, HDL cholesterol <1.03 mmol/L in men or <1.28 mmol/L in women, fasting blood glucose > 5.5 mmol/L and high blood pressure ≥130 mm Hg systolic or ≥85 mm Hg diastolic (Grundy et al,

2004). Based on this criteria 22 (32.8%) patients taking PI-based regimen has been found to have metabolic syndrome as compared to 12(17.9%) of those on NNRTI-based regimen, $X^2 = 3.941$, df =1, p = 0.047 (Figure 4.3).

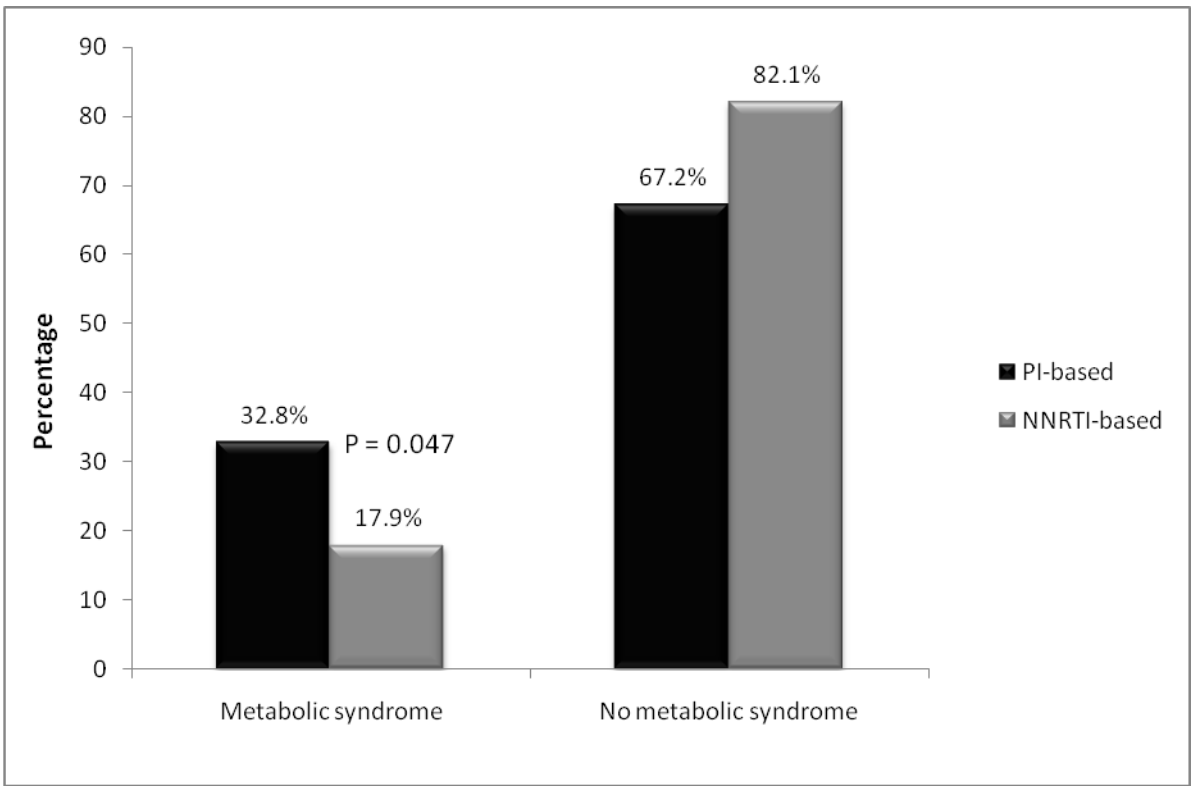

Figure 4.3 Prevalence of metabolic syndrome among patients taking PI-based and NNRTI-based regimen

4.15.10. Estimation of cardiovascular disease risk

The ten year cardiovascular disease risk estimate for the patients was carried out by the Framingham Risk Score which takes in to account the patients age, sex, smoking habit, systolic blood pressure as well as serum total and HDL-Cholesterol. According to this estimation, individuals with low risk have 10% or less Coronary Heart Disease (CHD) risk at 10 years, with intermediate risk 10-20% and high risk 20% or more (NCEP ATPIII update, 2004). Based on this scoring about 10.4% of patients on the PI-based regimen was found to have an intermediate risk or 10-20% chance of developing CHD in the coming ten years as opposed to only 1.5% of

patients on the NNRTI-based regimen (x^2 = 4.79, df = 1, P = 0.029) . However none of the patients in either regimen had estimated high risk.

Table 4.6 Comparison of absolute cardiovascular risk estimate between patients taking PI-based and NNRTI-based regimen.

Estimated 10 y CVD risk %	PI-based, n (%) n = 67	NNRTI-based, n (%) n = 67	P. value
<10% (low risk)	60 (89.6)	66 (98.5)	ns
10-20 % (moderate risk)	7 (10.4)	1 (1.5)	0.029
>20% (high risk)	-	-	-

CVD- Cardiovascular disease, ns- non-significant

4.15.11. Correlation analysis

4.15.11.1. Correlation of HOMA-IR with questionnaire variables, anthropometric measurements, blood pressure and biochemical variables among the cases.

According to Pearson's momentum correlation there was no statistically significant correlation of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) with age and duration of therapy. However, HOMA was found to be positively and significantly correlated with BMI (r = 0.37, p = 0.002), systolic blood pressure (r = 0.3, p = 0.014), diastolic blood pressure (r = 0.29, p = 0.017) and non-significantly negatively correlated with HDL (r = -0.022, p = 0.858)

Table 4.7 Correlation of HOMA with BMI, systolic BP, diastolic BP and serum HDL-C level among the cases

		BMI	Systolic BP	Diastolic BP	HDL-C
HOMA-IR	r	0.37	0.3	0.29	-0.022
	p. value	**0.002****	**0.014***	**0.017***	0.858

r- Correlation coefficient *p<0.05, **p<0.01

4.15.11.2. Correlation of triglyceride with questionnaire variables, anthropometric measurements and biochemical variables among the cases.

Again according to Pearson's momentum correlation coefficient there was no statistically significant correlation of serum triglyceride level with age and duration. However serum triglyceride level was found to be correlated with systolic blood pressure (r = 0.38, p = 0.001), diastolic blood pressure (r = 0.31, p = 0.01), BMI (r = 0.3, p = 0.014), WHR (r = 0.41, p = 0.001) , serum insulin level (r= 0.43, p<0.001) and serum total cholesterol (r = 0.51, p = <0.001). Triglyceride also showed a significant negative correlation with serum HDL-C level (r = -0.22, p = 0.011) (Figure 4.5).

Table 4.8 Correlation of TG with systolic and diastolic BP, BMI, WHR, serum insulin level and HDL-C level

		SBP	DBP	BMI	WHR	Insulin	HDL-C	TC
Serum triglyceride	r	0.38	0.31	0.3	0.41	0.43	0.22	0.51
	P. value	**0.001****	**0.01***	**0.014***	**0.001****	**<0.001****	**0.011**	**<0.001****

r- Correlation coefficient, SBP – Systolic blood pressure, DSP – Diastolic blood pressure, TC – total cholesterol *p<0.05, **p<0.01

4.15.11.3. Correlation of Body mass index (BMI) with Waist-to- hip ratio, systolic blood pressure and diastolic pressure among the case

Body mass index (BMI) also showed a moderate positive correlation with systolic blood pressure (r = 0.28, p = 0.02), diastolic blood pressure (r = 0.23, p = 0.058) and strongly positively correlated with waist to hip ratio (r =0.6, p <0.001) (Figure 4.4).

Table 4.9 Correlation of BMI with systolic BP, diastolic BP and waist to height ratio

Body mass index (BMI)		Systolic BP	Diastolic BP	WHR
	r	0.28	0.23	0.6
	p. value	**0.02***	0.058	**<0.001****

r- Correlation coefficient *p<0.05, **p<0.01

4.15.11.4. Correlation of absolute CD4 count with the serum biochemical variables

No significant correlation of absolute CD4 count has been found with either of the serum biochemical parameters assessed in this study. However, it was found to be non-significantly positively correlated with HOMA-IR, total cholesterol, LDL-Cholesterol and HDL-Cholesterol but non-significantly negatively correlated with serum triglyceride level.

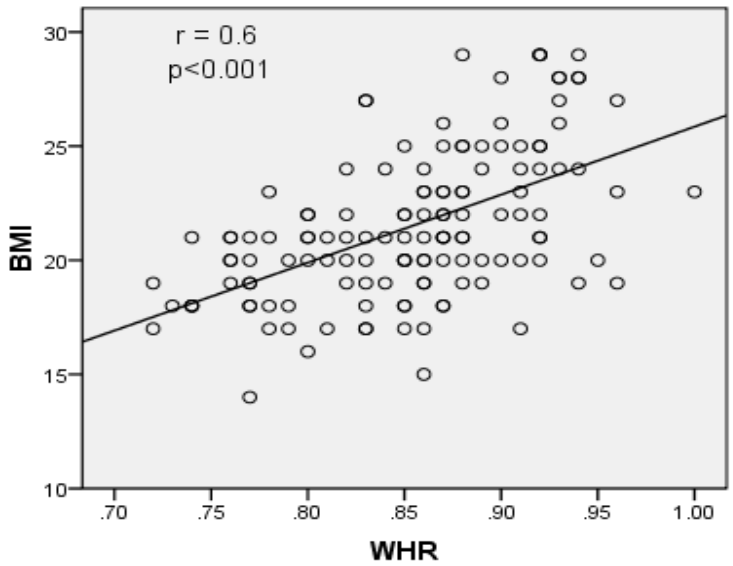

Figure 4.4 Regression fit of WHR versus BMI

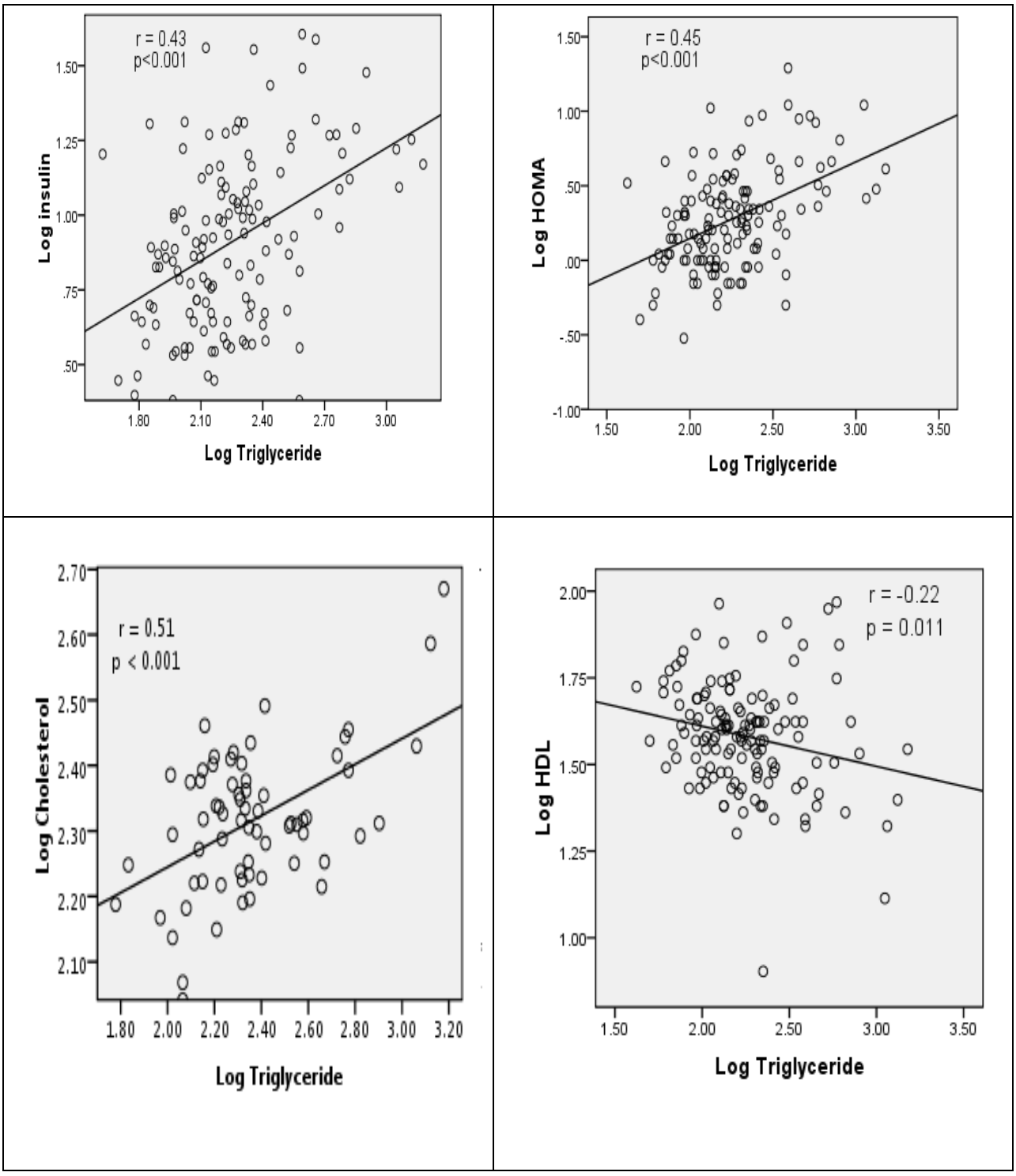

Figure 4.5 Regression fit of triglyceride versus Insulin, HOMA-IR, total cholesterol and HDL-C

5. Discussion

The study reported herein is aimed to assess insulin resistance and dyslipidemia in HIV-1 infected patients on PI based combined antiretroviral therapy as compared to controls that are on NNRTI-based therapy. The results show an elevation in serum triglyceride concentration and a trend toward increase in insulin resistance in patients treated with PI-based regimen, compared to NNRTI- based regimen.

Insulin resistance is an established adverse effect among HAART recipients, especially those who take a protease inhibitor (Babafemi, 2005). Initially all PIs were thought to cause IR as a direct class effect. However, the lack of an early increase in IR with Nelfinavir in one study suggested that this was not the case ((Dubé et al, 2004). Also the more recent PI, Atazanavir, does not seem to cause IR (Dubé et al, 2004). In agreement with this, another study declared that Atazanavir seems not to be associated with significant dyslipidaemia and insulin resistance as seen with other PIs (Piliero, 2002). Strengthening the drug specific effect of PIs, some other related but independent study also reported that Lopinavir/ritonavir induces significant hypertriglyceridemia under conditions where it has little effect on insulin resistance (Lee, 2004).

The current study showed a non-significant trend in elevation of insulin resistance among the PI-based regimen, 34.3% as compared to 28.4% in NNRTI-based regimen. The proportions of patients with insulin resistance and mean HOMA values however did not significantly differ between PI- and NNRTI-based, nor did fasting insulin levels. The 28.4% insulin resistance among those on NNRTI-based cART is somewhat higher than the 18% claimed for HIV-negative subjects (Bergersen et al, 2006) but concords with most researchers who reported an insulin resistance in the range of 20% - 35% for patients taking NNRTI-based regimen (Walli et al, 1998; Carr et al, 1999; Gobel et al, 1999; Babafemi, 2005 and Aboud et al, 2007). In contrast to this, the 34.3% insulin resistance observed in our patients on PI-based regimen is much lower than the prevalence's ranging 47-85% published in most literatures for the same group (Walli et al, 1998; Carr et al, 1999; Gobel et al, 1999; Babafemi, 2005 and Aboud et al, 2007). This variation could be, among others due to the difference in PIs used in the respective studies, where they considered Indinavir based HAART as opposed to Lopinavir/ritonavir based HAART usage in our study.

Patients with insulin resistance tend to remain euglycemic as long as there is an adequate compensatory increase in insulin output from the pancreas. Impaired glucose tolerance and diabetes ensue when the level of insulin resistance exceeds the compensatory increase in pancreatic insulin output (Babafemi, 2005). Supporting this idea, Elevated glucose levels were apparent in a small proportion of our study population, in 14.9% of patients on PI-based regimen and in 19.4% of patients on NNRTI-based regimen with the means for both in the normal range. This result is in agreement with most studies which reported a prevalence of hyperglycemia ranging from 0–20% during PI-containing HAART therapy (Dali et al, 2002). The prevalence in the NNRTI-based group is also concordant with the 14% hyperglycemia observed in patients on nucleoside reverse transcriptase inhibitors (NRTI) treatment (Galli et al, 2002).

Metabolic syndrome, involves the coexistence of elevated blood pressure, dysfunctional glucose homeostasis, obesity and dyslipidemia, owing to the effects of insulin resistance on insulin's target organs (Babafemi, 2005). In the present study a significantly higher proportion (32.8%) of patients on PI-based regimen were found to have metabolic syndrome as compared 17.9% in the NNRTI-based regimen. Individuals with metabolic syndrome are at increased risk for coronary heart disease (CHD). In Framingham, the metabolic syndrome alone predicted ≈25% of all new-onset cardiovascular diseases (CVD) (Grundy et al, 2004). In the current study one tenth (10.4%) of the patients on PI-based regimen was found to have 10-20% (intermediate risk) chance of developing cardiovascular risk in the coming ten years as compared to only 1.5% in the NNRTI-based regimen. Other studies also reported that hypertension is frequent in HIV patients on HAART and that it appears to be linked to insulin resistance (Gazzaruso et al, 2003). In line with this, HOMA showed a positive correlation with body mass index, waist to hip ratio and blood pressure in the present study.

Inhibition of LDL receptor related protein (LRP) results in lower uptake of triglycerides by the liver and reduced cleavage of fatty acids and glycerol, which should occur through LRP-LPL (lipoprotein lipase) complex activity (Leonardo et al, 2003). Hypertriglyceridemia also accounts for the increased insulin resistance, which can lead to diabetes mellitus type II (Behrens et al, 2005). Moreover, insulin resistance correlates closely with abdominal obesity and hypertriglyceridemia and underlies diabetes mellitus type II (Matsuzawa et al, 1995**,** Carey et al, 1996**)**. In our study HOMA-IR showed a statistically significant positive correlation with serum

triglyceride level, body mass index and non significant positive correlation with waist to hip ratio. This could partly explain the non-significant trend in elevated prevalence of insulin resistance among the cases as compared to the controls.

Abnormal glucose metabolism is exacerbated by a family history of diabetes, combined with HAART and overweight (Blass et al., 2008). In agreement with this report, our study revealed that 6 of the 19 patients (31.6%) with family history of diabetes mellitus had hyperglycemia as compared to only 17 of 115 patients (14.8%) among those who do not have the genetic predisposition (P = 0.11). This shows a trend towards elevated hyperglycemia among the PI-treated even if not to statistically significant level.

HIV-infected patients were already known to be at risk of dyslipidemia before the HAART era (Grunfeld et al, 1992). Introduction of HAART has brought this problem to the forefront with the potential of impeding prolonged survival by increased cardiovascular events (Grunfeld, 2000). The impact of HAART on biochemical parameters has been widely published and many studies have shown that PI-based HAART has a negative impact on blood lipid levels (Montessori et al, 2004; Wanke et al 2004; Grinspoon, 2005, Stein, 2009). However, the prevalence of dyslipidemia varies according to the type of drug used by the patients (Calza et al, 2003). Several studies have shown a significant increase in triglycerides and total cholesterol on LPV/r-based therapy. In a population of 74 previously treatment naive HIV infected patients, Magenta et al. found that most of the lipid and lipoprotein plasma levels increased after initiation of a LPV/r based cART (Magenta et al, 2003). When time on treatment was considered most of these increases tended to occur during the first year of cART and reached a plateau in the following years. This is consistent with our study that serum triglyceride level was not significantly correlated with duration of HAART.

According to Shah et al. (2005), lipid profiles should be checked regularly in patients on HAART, specifically individuals on PI therapy, as high rates of dyslipidemia occur in this population group. Protease inhibitors are generally associated with elevated levels of triglycerides and total cholesterol. Triglyceride levels of greater than 1,000 mg/dL have been reported in association with protease inhibitors (Sullivan et al, 1997, Carr et al, 1999). This may, at least in part be, because protease inhibitors reduce triglyceride deposition in adipose tissue and

limit their clearance from the circulation by lipoprotein lipase through binding and inhibition of CRABP-1 and LRP respectively(Nageswara et al, 2002; Leonardo et al, 2003 and Piliero, 2003). Moreover, they also inhibit apolipoprotein B catabolism in the liver there by increase VLDL production. The elevation in total cholesterol and LDL level could also be due to protease inhibitor induced mitochondrial dysfunction a feature which is also shared by NRTIs.

In this study, a significantly higher proportion of patients on PI-based regimen presented with dyslipidemia (elevated triglyceride levels, elevated total cholesterol to HDL ratio and TG/HDL ratio) and non-significant trend of low HDL-C when compared to patients on NNRTI-based regimen. This is in accordance with the results found by Shah et al (2005) and Wanke et al (2005), among HIV infected patients on HAART in Texas and Boston, respectively. Hypertriglyceridemia was the most prevalent and found in nearly three-fourths of patients (74.6%) on PI-based regimen as compared to 34.3% on non PI-based regimen. The second most common abnormality was LDL-C (65.7%) followed by hypercholesterolemia (58.2%) affecting more than half of the studied subjects. These findings are consistent with previous studies which showed that the prevalence of dyslipidemia in patients receiving PI-containing ART ranged from 28-80%, of which the majority were hypertriglyceridemia (40-90%) and hypercholesterolemia (10-50%) (Behren et al, 1999; Carr et al, 1999; Mercie et al, 2000; Thiebaut et al**,** 2000; Tsiodras et al, 2000; Benesic et al, 2003; Farris- Moller et al, 2003; Tomazic et al, 2003; Alain et al, 2004,).

Hypertriglyceridemia appears to involve in cardiovascular risk in several ways. It is the primary driver of the metabolic disarrays which results in low HDL-C and small, dense LDL (sdLDL) that characterizes atherogenic dyslipidemia and plays a major role in overall cardiovascular risk. In hypertriglyceridemic states, triglyceride enrichment of HDL resulting from cholesteryl ester transfer protein-mediated exchange with triglyceride rich lipoproteins enhance the lipolytic transformation and subsequent metabolic clearance of HDL particles thereby leading to impaired reverse cholesterol transport (Thomas et al, 2006). Strengthening this concept, the highest percentage of hypertriglyceridemia observed in the PI-based subjects in our study was also accompanied by concomitant lowering of HDL-C.

The ratio of TG/HDL-C, initially proposed by Gaziano et al (1997) is an atherogenic index that has proven to be a highly significant independent predictor of myocardial infarction, even stronger than TC/HDL-C and LDL-C/HDL-C (Protasio et al, 2008). Studies have also demonstrated that the elevated TG/HDL-C (>3.8) would suggest the predominant LDL particle size is small in 79% of the patients (Protasio et al, 2008). Moreover small LDL particles have an increased susceptibility to oxidative modification and oxidized LDL contributes to the development of atherosclerotic lesions in several ways, including accumulation in macrophages to form foam cells and modulation of various pro-inflammatory pathways. Therefore, further exacerbating the cardiovascular risk among the PI-based group, the current study also revealed that a significantly higher proportion of PI-treated (74.6%) had a TG/HDL ratio >3.8 as compared to 38.8% among the NNRTI-treated subjects. Of note, the significant elevation of the aforementioned atherogenic indices together with a marked rise in the total cholesterol to HDL ratio (61.2 versus 40.3 among the PI and NNRTI-based respectively) clearly shows that HIV patients treated with PI-based HAART have a greater risk of developing cardiovascular problem.

Excessive weight gain and the incidence of obesity and overweight are increasing in the HIV infected population on HAART (Bhavan et al., 2008). HIV infected patients on HAART that are overweight have a higher risk for cardiovascular disease (Kaplan et al, 2007). In the present study, the results indicated that the majority of the patients were within the normal weight range according to BMI. More patients, however, were overweight on PI-based regimen than on NNRTI-based regimen (28.4% versus 10%).

The current study also revealed that serum triglyceride level was positively and significantly correlated with body mass index, waist to height ratio, systolic and diastolic blood pressure, serum insulin level and negatively correlated with serum HDL level. This supports the idea that dyslipidemia is linked to insulin resistance and fat redistribution syndromes (Grover et al, 2005; Oh and Hegele, 2007). BMI was also significantly and positively correlated with waist to height ratio and blood pressure thus increasing the cardiovascular disease risk (Tomazic et al., 2004).

6. Conclusion

This study has revealed a trend toward elevation in the proportion of patients developing insulin resistance and dyslipidemia in the PI based regimen as compared to the NNRTI-based regimen with statistically significant elevations in triglyceride, total cholesterol to HDL ratio and triglyceride to HDL ratio. Moreover, the prevalence of metabolic syndrome was higher in this group with an increased estimated cardiovascular disease risk. The metabolic disease risk indicators like body mass index, waist to height ratio and blood pressure were also significantly and positively correlated with each other and with triglyceride and HOMA-IR value. Furthermore, Serum triglyceride level was positively and significantly correlated with both serum insulin level and HOMA-IR. It is understood that beyond its cardiovascular risk, hypertriglyceridemia has an added effect as a major predisposing factor for insulin resistance. Thus, In conclusion, this study has indicated that patients on PI-based regimen have a greater risk of developing diabetes mellitus and cardiovascular disease risk as compared to those on NNRTI- based regimen.

7. Limitations of the study

The major short coming of this study is that the study participants feeding and drinking habits were not assessed, which independently can contribute for the metabolic derangements observed in the patients. Another limitation of the study is that the serum level of non esterified fatty acids was not measured.

8. Recommendations

- Though the role of protease inhibitors in suppressing HIV viremia cannot be questioned, the associated biochemical alterations observed in this and similar other studies may compromise their role in changing HIV from a catastrophic disease to a chronic manageable disease. Therefore the serum biochemical parameters of patients taking HAART, in particular PI-based regimen should be regularly followed as an integral part of their management.

- In our study patients on the PI-based (2^{nd} line) regimen was found to be more at risk of developing diabetes and cardiovascular disease than those on NNRTI-based (1^{st} line) regimen. Therefore, greater effort is required by health professionals to create awareness on the patients concerning treatment adherence so as to prevent drug resistance and retain them on the first line.

- Despite the higher proportion of individuals with insulin resistance observed in the PI-based group as compared to the NNRTI-based, the difference was not statistically significant. Therefore, further study should be conducted with more study participants and more parameters assessed to make the findings more representative and confirm whether the current result is most likely or happened by chance.

- Lastly but not least, the adverse effect of Lopinavir/ritonavir on hypertriglyceridemia revealed in our study insist us to urge the policy makers to look for other replacement PIs like Atazanavir which has little documented adverse effect.

9. Annexes

Annex I

Information sheet and consent form for participants of the study entitled" insulin resistance, dyslipidemia and cardiovascular disease risk in HIV-1 infected adults receiving PI-based combined antiretroviral therapy in the ART clinic".

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A. Information sheet (English version)

This information sheet is developed by the aforementioned principal investigator at AAU for a project with the aim of assessing insulin resistance, dyslipidemia and Cardiovascular disease risk in HIV-1 infected patients receiving PI-based antiretroviral therapy. You are being cordially invited to take part in the study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to listen the following information carefully and discuss it with others if you wish. You have the chance to seek for more explanation by the principal investigator for any vague point. Finally you will have ample time to think and decide whether or not you wish to take part.

Aim of the study

Protease inhibitors are among the antiretroviral drugs which help HIV infected individuals by reducing viral load, improving CD4 count and thereby improve their survival**.** Besides improving patient prognosis, these drugs also result in metabolic abnormalities like insulin resistance and dyslipidaemia. Consequently, patients are also at high risk of developing type 2 diabetes mellitus and premature cardiovascular morbidity. Therefore the plan of this study is to assess the prevalence of insulin resistance and dyslipidemia in HIV-1 infected patients receiving PI-based antiretroviral therapy and to make recommendations accordingly.

Study design and procedure

If you agree to participate in the study, investigators will give you verbal and/or written information about the study and you will be given the consent form to sign. The investigators will ask you some information about your general health and will take anthropometric measurements so as to decide whether you qualify for the study or not. If you are fit for the study you will be requested to fast over night and give about 5 ml of blood required to conduct the study.

Risk and discomfort

Participating in this project will not cause more discomfort and do not need extra volume of sample than that required for routine diagnostic purpose. However, there could be minor pain during blood drawing which disappear within a moment. The 5 ml amount of blood taken will not affect your health and there is no major risk in participating in study as the whole procedure is going to be carried out by health professionals in accordance with the standard procedure.

Benefits and incentives

You will not be provided with any direct incentive but you will have the chance to know your fasting blood sugar, insulin level and lipid profile from the laboratory results in which their cost is covered by the project. In addition if your results show any indication that need immediate management or continuous follow up, it will be communicated with your physician so that you will be managed accordingly. Above all the study outcome will benefit all HIV patients receiving PI-based regimen.

Confidentiality

All information obtained during the course of this study will be kept confidential and will be disclosed only with your permission if at all.

Right to refuse or withdraw

Please be aware that your participation in this study is entirely voluntary and you have full right to refuse or withdraw at any point before and after consent without reasoning. Be sure that your decision will not affect your right to take medication or any other health service now or in the future.

Annex II

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Annex III

B. Consent form

By my signature below, I confirm that I have been provided with and read this informed consent. I understand that this is a research study and my participation is voluntary with full right to terminate at any point without any consequence with regard to my medical care or legal rights. I have had full explanation about the purpose of the study, the procedures, risks and benefits and the opportunity to ask questions and my questions have been answered. Therefore my participation is voluntarily and I give permission for the investigator to have access for my information and take blood by signing on this consent form.

Patient code number	**date**	**signature**
Principal investigator	**date**	**signature**

Annex IV

______________________ ______________________ ______________

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Annex V

Questionnaire (English version)

Data collection Questionnaire for the comparative study on insulin resistance, dyslipidemia and cardiovascular risk in HIV-1 infected adults receiving PI-based versus non PI based combined antiretroviral therapy among patients attending at Black lion Specialized Hospital ART clinic, Addis Ababa, Ethiopia.

Date of patient Visit---------- patient code number ----------

Please answer all questions honestly; you will not be "judged" based on your responses. Your specific responses to questions will not be reviewed. Please feel free to ask if you need any of the questions explained to you.

I. Socio-demographic characteristics

1. Age _____
2. Sex: Male_____ female_____
3. Marital status

   Single_____ Married_____ divorced _____ widowed_____ under age_____

4. Occupation

   Gov't employee _____ private employed___ Unemployed_____ Others___

5. Level of education

   Illitrate ___ Elementary school ___ Secondary school ___ High school _____ College graduate ___ University graduate ____

II. Anthropometric measurements

1. Current weight ____Kg Current height _____cm Body mass index (BMI) _____kg/m^2
2. Hip circumference _____cm Waist circumference _____cm Waist to hip ratio _____
3. Blood pressure: Systolic/diastolic (_____/_____)mmHg

III. Others

6. Which regimen are you taking currently?

   A. 1st line_____ B. 2nd line _____ Other_____

7. For how long you been on this regimen? _____(months/years)
8. Are you pregnant (for female participant)?

   A. Yes_____

   B. No_____

9. Are you currently lactating (for female participant)?

   A. Yes_____

   B. No_____

10. Do you have diagnosed diabetes mellitus?
    A. Yes _____ go to no 11
    B. No _____
11. When did you diagnosed?
    A. Before initiation of ART _____
    B. After initiation of 1st line regimen _____
    C. After switching in to 2nd line regimen _____
12. Have you ever had insulin test?
    A. Yes _____ go to No 13
    B. No _____
13. What was the result? _____
14. Is there any family member with diabetes mellitus?
    A. Yes_____
    B. No_____
15. Have you ever had gestational diabetes (for female participant)?
    A. Yes_____
    B. No_____
16. Do you have diagnosed cardiovascular disease?
    A. Yes _____ go to no 17
    B. No _____
17. When did you diagnosed?
    A. Before initiation of ART _____
    B. After initiation of 1st line regimen _____
    C. After switching in to 2nd line regimen _____
18. Are you currently taking any medication other than ART?
    A. Yes _____ go to No 19
    B. No _____
19. What type (specify)_____
20. Does your job need moderate physical activity?
    A. Yes_____
    B. No_____
21. Do you have smoking habit?
    A. Yes_____
    B. No_____

THANK YOU FOR YOUR COOPERATION!

Annex VI

Amharic Version Questionnaire

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Annex VII

Declaration

I, the undersigned, declare that this M.Sc. thesis is my own original work, has not been presented as thesis work for a degree in this or other University and that all sources of materials used for this thesis have been duly acknowledged.

Investigator Hagos Amare (B.Sc)

Signature _____

Date of submission _____

Supervisors:

1. Daniel seifu (PhD)

   Date_____ Signature_____

2. Aster Tsegaye (phD)

   Date_____ Signature_____

3. Wondwossen Amogne (MD, Internist)

   Date_____ Signature_____

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