Determination of efavirenz based Antiretroviral Therapy associated Insulin Resistance in adult Human Immunodeficiency Virus infected patients receiving Antiretroviral Therapy alone and in combination with Anti Tuberculosis drugs in Addis Ababa.

Kaleab Gizaw

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This is to certify that the thesis prepared by Kaleab Gizaw entitled: "*Determination of efavirenz based Antiretroviral therapy associated insulin resistance in adult Human Immunodeficiency Virus infected patients receiving Antiretroviral therapy alone and in combination with Anti Tuberculosis drugs in Addis Ababa"* and submitted in partial fulfillment of the requirements for the Degree of Master of Science complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Determination of Efavirenz based Antiretroviral Therapy associated Insulin Resistance in adult Human Immunodeficiency Virus infected patients receiving Antiretroviral Therapy alone and in combination with Anti Tuberculosis drugs in Addis Ababa.

Kaleab Gizaw

Addis Ababa University, 2019

The invention of Highly Active Antiretroviral Therapy (HAART) has achromatized the Human Immunodeficiency Virus (HIV) infection from a fatal disease to a chronic condition marked by reduced morbidity and mortality. This success in controlling HIV replication and restoring immunity has been displeasured by the acclamation that metabolic changes, such as Insulin Resistance (IR) are increasing among people living with HIV. The goal of this study was to determine IR and assess risk factors associated with it among HIV infected patients receiving efavirenz (EFV) based antiretroviral medications alone and in combination with rifampicin (RIF) based anti-Tuberculosis (TB) medications. A prospective and comparative two arm cohort study of 30 HIV infected patients on HAART and 30 TB-HIV coinfected patients on HAART and Anti-Tuberculosis drugs was employed. Blood samples of patients with HIV infection and TB-HIV co-infection were identified and compared for presence of insulin resistance. Association between insulin resistance and risk factors was analyzed. The Mean Triglyceride to Glucose (TyG) index, the measure of IR, in HIV infected groups receiving EFV based HAART alone and TB-HIV co-infected groups receiving EFV based HAART in combination with RIF based anti TB, was not significantly different. Baseline plasma triglyceride was associated with elevated TyG index and IR (OR, 95% CI = 1.170; $p < 0.01$) in the TB-HIV co-infected groups. In both treatment groups, a significant mean difference in IR was observed in patients with an advanced HIV infection**.** In HIV only treatment group, a higher mean TyG was found among stage 4 patients at week 4 ($p = 0.034$) and week 16 ($p = 0.049$); and in TB - HIV cotreatment groups, a higher mean TyG index was found among stage 4 patients at week 16 ($p = 0.019$). No significant difference in insulin resistance was observed between HIV patients receiving EFV based Antiretroviral Therapy (ART) alone and TB-HIV co-infected patients receiving EFV based ART with RIF based Anti TB medications. EFV based HAART can lead to insulin resistance independent of HIV and non-HIV related risk factors.

Key words: *Insulin Resistance, Efavirenz, Antiretroviral therapy, Rifampicin, TyG index, Ethiopia*

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List of abbreviations / acronyms

AIDS	Acquired Immune Deficiency Syndrome
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
ART	Anti-Retroviral Therapy
AST	Aspartate Aminotransferase
DM	Diabetes Mellitus
EFV	Efavirenz
HAART	Highly Active Anti-Retroviral Therapy
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
IR	Insulin Resistance
LDL	Low Density Lipoprotein
RIF	Rifampicin
TB	Tuberculosis
TG	Triglyceride
TyG	Triglyceride to Glucose index
T2DM	Type 2 Diabetes Mellitus

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1. Introduction

1.1 Background of the study

Huge efforts to control the Human Immunodeficiency Virus (HIV) were implemented since the initial description of the Acquired Immunodeficiency Syndrome (AIDS) and extended after the isolation of HIV-1. The initial years were unsuccessful and characterized by treatment failure and disappointments. The development of a diagnostic antibody test, however, was the life changing phenomenon. Following this, clinical trials began with direct acting dideoxy Nucleoside Reverse Transcriptase Inhibitors (NRTIs), the first being Azidothymidine (AZT) (Stefano et al., 2012).

Despite its short lived benefits and side effects, AZT, later called zidovudine (ZDV), was approved for use. In short succession, three other NRTIs were approved for use in HIV-1 infection: zalcitabine (ddC), didanosine (ddI) and stavudine (d4T). By making dual combination NRTI therapy as an advanced approach, encouraging results were observed where, the impact in terms of CD4β lymphocyte increase and survival was better. Results, however, were still not durable (Stefano et al., 2012). .

A step forward occurred when the cytidine analog Lamivudine (3TC) was developed. The drug showed synergistic effect when given with any of the other nucleosides including ZDV and was relatively well tolerated however, monotherapy was associated with the rapid development of resistance. Despite this entire attempt, it was found that none of the dual nucleoside combinations, when administered without a third drug, were able to durably control HIV infection (Kuritzkes et al., 1999). Therefore, the need to use triple NRTIs to control the virus became the next target. The first trial to demonstrate this was the Italy, Netherlands, Canada, Australia Study (INCA Study), which showed triple nucleoside therapy to be completely suppressive and superior to dual nucleoside therapy (Montaner et al., 1998).

In the following years, many drugs, having a direct effect on HIV were developed. For instance, in 1998 a new NNRTI, Efavirenz (EFV), was approved for use in combination with other regimens. The next step became the combined use of drugs from different classes. Two years later, the first description of metabolic toxicities of ART was reported (Stefano et al., 2012).

Despite the role of ART in improving patient's life expectancy, new challenges including cardiovascular and metabolic diseases such as Diabetes Mellitus (DM) started to emerge (Grinspoon et al., 2005).

Diabetes Mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia where the hyperglycemia can be due to defects in insulin secretion, insulin actions or both. It is associated with reduced life expectancy, significant morbidity due to specific diabetes related microvascular complications, increased risk of macrovascular complications and diminished quality of life.

According to the American Diabetes association, DM is classified into four general classes as Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), Gestational diabetes mellitus (GDM) and other Specific types of diabetes due to other causes where drug or chemical-induced diabetes are grouped in the latter and drugs used in the treatment of HIV/AIDS were taken as an example (Figure 1) (American diabetes association, 2017).

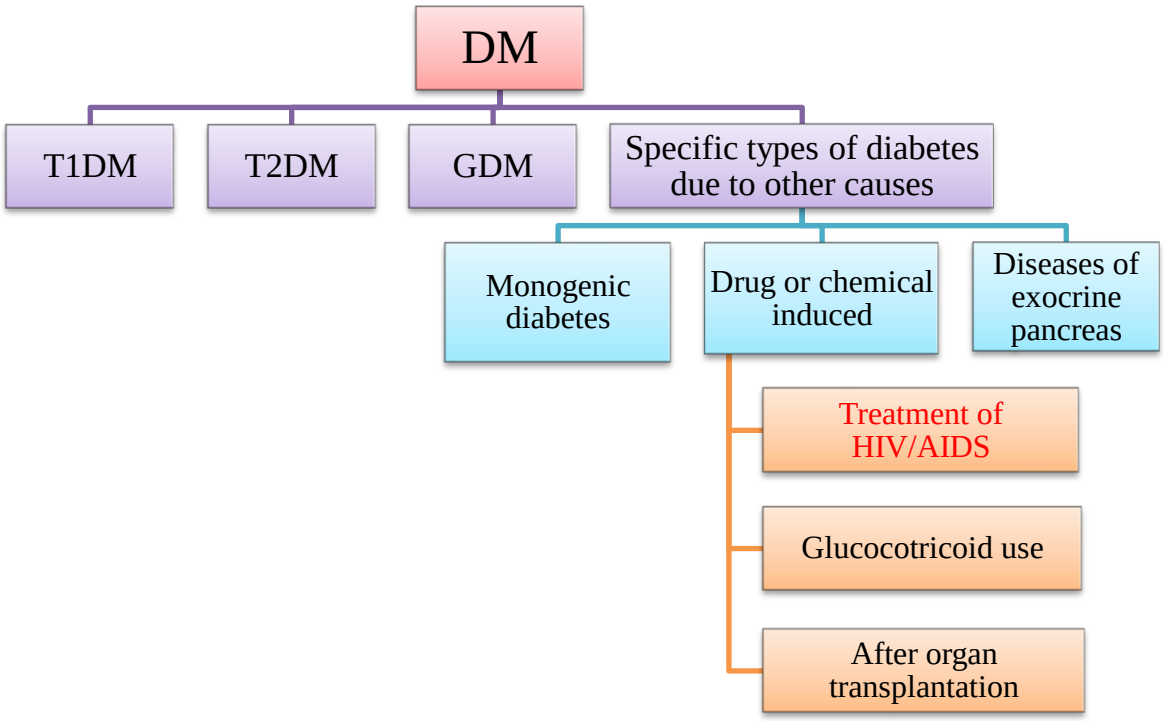

Figure - 1 Classification of diabetes according to American Diabetes Association, 2017

With a high prevalence of diabetes in the background population, it stands to reason that the same predisposing factors will operate in patients with HIV. In addition to this, certain metabolic factors related to HIV, and to HIV therapy, were believed to increase the incidence of diabetes (Kalra et al., 2011). A recent analysis has found diabetes to be fourfold more common is HIV infected men exposed to HAART than HIV seronegative men (Brown et al., 2005).

The major predisposing factor for DM, especially for T2DM is Insulin Resistance (IR). IR is defined as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and utilization in an individual as much as it does in a normal population. In clinical practice, it refers to a state in which a given concentration of insulin is associated with a subnormal glucose response (Moller et al., 1991).

Patients infected with HIV/AIDS are highly prone to developing insulin resistance than HIV uninfected patients. A comparative study conducted in the US aiming to study the relation between ART exposure and IR in HIV infected and uninfected women found that, HIV infected women who reported using any HAART regimen at the last visit had greater IR than HIV uninfected women (Phyllis et al., 2008).

Similar to this, few studies found an association between tuberculosis and IR development in newly diagnosed pulmonary tuberculosis patients with a prevalence of 25.4% (Lauren et al., 2017). The association between Tuberculosis and anti TB drugs with IR and DM is presented in the literatures review section in detail.

Insulin resistance involves inhibition of insulin stimulation of several metabolic pathways including glucose transport, glycogen synthesis and anti-lipolysis. Owing to such effects, it is of considerable clinical relevance and is pathophysiologically linked to several serious medical problems including hypertension, coronary artery disease and polycystic ovarian syndrome in addition to DM (Reaven, 1988).

1.2 Statement of the problem

The HIV pandemic has continued to spread worldwide. The number of people living with HIV/AIDS was 36.9 million and the number of people newly infected with HIV was 1.8 million globally in 2017. The burden of the disease is high in sub-Saharan Africa, where 25.7 million (69.64%) adult and child infections and 1.17 million (65%) new infections were recorded by the end of 2017 (UNAIDS, 2018).

The total number of people in Ethiopia who are living with HIV/AIDS in 2017 was around 610,000. Around 16,000 new infections were recorded in that year (UNAIDS, 2018). This number is expected to increase to 754,256 by the year 2021 (Ethiopian Public Health Institute, 2017).

The consumption of antiretroviral drugs increased from 7.5 million in 2010 to 17 million in 2015 (Global AIDS Response Progress Report, 2016) which then increased to 21 million in 2017 (UNAIDS, 2018). In 2017, 21.7 million people were accessing ART where 15.3 million (70.50%) ART users reside in sub-Saharan Africa, making them the leading ART consumers in the globe (UNAIDS, 2018).

In 2011, there were an estimated 222,723 people receiving antiretroviral therapy in Ethiopia (WHO, Global AIDS response, 2011). Ethiopia became one of the 25 Fast-Track countries in the distribution of ART in 2015 (Global AIDS Response Progress Report, 2016). In 2017, the total number of patients accessing ART was 389,766 with a prevalence of 71% (UNAIDS, 2018).

Global scale-up of ART has been the primary contributor to a 48% decline in deaths from AIDS related causes in 2016 and for the first time, more than half of all people living with HIV (53%) were accessing ART (Global AIDS update, 2017). Therefore it can be said that the introduction of ART substantially improved the prognosis of HIV/AIDS patients, with a reduction in morbidity and mortality due to opportunistic diseases and consequent improvement of the patient's quality of life (Lewden et al., 2012).

These advantages of HAART in controlling HIV replication and restoring immunity have come at the price of increased incidence of unanticipated adverse metabolic effects, including insulin

resistance, diabetes, dyslipidemia, and lipodystrophy (Schambelan et al., 2002, Guiterrez et al., 2007, Kalra et al., 2011 and Calmy et al., 2007).

The clinical presentation of antiretroviral-associated diabetes is consistent with that of type 2 diabetes. In one study, insulin resistance, rather than insulin deficiency, was reported to be usually implicated in the pathogenesis of diabetes in HIV infected patients and evidence of islet cell autoimmunity or beta cell destruction has not been seen in HIV patients (Brown et al., 2005).

It is projected that the number of people affected by DM will increase to 592 million by 2035 (International Diabetes Federation, 2015) and about 80% of these people live in low- and middle-income countries where infectious diseases such as TB and HIV/AIDS are endemic (WHO Global tuberculosis report, 2015).

Currently, Ethiopia is also facing an increasing rate of DM among its population. With 3.8% prevalence among its population, Ethiopia is the third highest country in Africa in terms of DM burden (WHO, Diabetes country profiles, 2016). It is estimated that the country will take the second place by the year 2030.

Even though DM and other metabolic complications are impending challenges in countries with high HIV prevalence, very little evidence exists on the disease burden of DM in the HIV infected population in the study area. In addition, there are scarce data about the incidence and determinants of diabetes among HIV infected adults in this region.

The trend of diabetes in HIV patients of Ethiopia is not well documented and its national prevalence is not known, although there are few studies that are conducted in localized areas. For instance, a study conducted at the University of Gondar indicated an 8% prevalence of diabetes among patients taking ART (Abebe et al., 2016).

To the knowledge of the researcher, there are no published articles that associate the use of rifampicin based anti TB medications with the development of IR and DM. Few studies, however, reflected DM as a predisposing factor in the development of active TB and signify the prevalence of TB where the DM burden is higher (Workneh et al., 2016).

Despite the fact that various published literatures tried to show the association between ART use, specifically protease inhibitors with impaired glucose tolerance, impaired insulin sensitivity and

in some case development of DM; few other studies suggested the role of other ART agents in causing the same problem. But there were very scarce data that associate the risk of DM with the use of EFV based regimens in the study area. In addition, there was no comparative study that showed the risk of IR or DM among HIV infected and HIV-TB co infected individuals.

It is hoped that findings of this study will add new evidences for the rising rate of DM in the country which might partly be due to ART regimens. Similarly, this study will come up with new insights regarding the risk of IR in HIV only infected and TB-HIV coinfected patients on efavirenz based antiretroviral regimen since most patients these days are on this regimen and less is known about its role in insulin resistance. The findings could also be used for preventive care of HIV/AIDS patients, thereby reducing incidence and severity of diabetes. Furthermore, the findings from this study are believed to reduce/prevent development of metabolic complications and drug induced mortality among people living with HIV/AIDS.

In addition, results of this study will be used as a source of data for hospitals, health centers, regional and national health bureaus and the Ministry of Health. Similarly, other Non-Governmental Organizations and stakeholders will use these data for their program draft and implementation. These data will also help researchers undertake further investigations.

1.3 Literature review

1.3.1 Pathogenic mechanisms and risk factors of insulin resistance/diabetes mellitus

Different pathogenic mechanisms of IR have been explained in both HIV infected and HIV uninfected individuals. These include genetic influences, elevated circulated free fatty acids, increased muscle and organ fat, hormones, co-morbid diseases and chronic inflammatory changes. But, the two major risk factors deemed to be responsible for the development and progression of IR that are specific for HIV patients, are lipodystrophy and antiretroviral medications, specifically Protease Inhibitors (Diana et al., 2007).

Increased accumulation of visceral fat, with wasting of subcutaneous fat, noted in HIV patients, was believed to create higher levels of inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF α). This in turn led to diabetes or impaired glucose tolerance by increasing insulin resistance (Kalra et al., 2011).

Other risk factors that contribute to the development of insulin resistance in HIV patients includes HIV itself, socio-demographic factors, HIV infection related factors, co-morbid conditions, concurrent medications and development of metabolic syndrome.

A multi-centered AIDS cohort study that assessed the prevalence and incidence of DM discovered the prevalence in HIV infected patients not using HAART to be 7%, indicating the role of HIV itself on development of DM (Brown et al., 2005).

In another study, IR was associated with HIV infection itself in patients not receiving HAART therapy. In that study, the IR was believed to result from the direct effects of the HIV virus on pancreatic betta cell function and insulin secretion (Dube, 2000).

Socio-demographic factors such as advancing age (greater than 40), male gender, higher BMI, lower socio-economic class, and certain ethnic backgrounds or culture are also risk factors for development of IR and DM (Ledergerber et al., 2007, De wit et al., 2008, Phyllis et al., 2008 and Kalra et al., 2011).

Viral factors which contribute to diabetes risk are an increase in viral burden of 0.5 log over a six month period, a lower CD4 count (below 200 cells/mm^3) and longer duration of HIV infection

(Fitchenbaum et al., 2005, Kalra et al., 2011 and Gutierrez et al., 2012). In general, people with severe, long-standing HIV infection were more prone to developing diabetes.

HIV infection is linked with hepatitis C infection (HCV), which is associated with insulin resistance and diabetes, due to increased intra hepatic tumor TNF α and hepatic steatosis. These factors increase the risk of diabetes in a patient suffering from concurrent HIV and HCV infection. Persons with HCV who are 40 years of age or older are greater than 3 times more likely to have diabetes than those of the same age without HCV infection (Mehta et al., 2001).

Concurrent use of medications was associated with IR in the general population including opiates and heroin. However, specific to HIV patients, drugs that are given for the management of opportunistic infections were studied and were found to be associated with insulin resistance. Pentamidine, used for prevention of Pneumocystic Carini Pneumonia, is believed to cause β-cell toxicity, with acute hypoglycemia followed by later diabetes (Sands et al., 1985).

In addition, metabolic syndrome is a more specific and an independent risk factor for development and progression of insulin resistance among HIV patients. An international cross-sectional study of 788 HIV infected adults recruited at 32 centers has studied the metabolic syndrome prevalence using International Diabetes Federation (IDF) and U.S. National Cholesterol Education Program Adult Treatment Panel III (ATPIII) criteria and discovered the prevalence of metabolic syndrome to be 14% by IDF criteria and 18% by ATPIII criteria. Type 2 diabetes prevalence was five to nine folds higher in those with metabolic syndrome (Kalra et al., 2011).

1.3.2 Prevalence of insulin resistance and diabetes mellitus

A Multicenter AIDS Cohort Study showed that 14% of HIV infected men using HAART at the baseline visit had prevalent DM compared with 5% of HIV seronegative men. The rate of DM incidence was 4.7 cases per 100 person-years among HIV infected men using HAART compared with 1.4 cases per 100 person-years among HIV seronegative men. The incidence of DM in HIV infected men with HAART exposure was greater than 4 times that of HIV seronegative men (Brown et al., 2005).

A study conducted by Susana et al. discovered the prevalence of IR among patients taking ART to be 21%**,** where 6% of the IR was observed in patients taking first-line regimens including efavirenz (Susana et al., 2014).

Another study that assessed the prevalence of IR and dyslipidemia in HIV infected patients who were on ART showed the prevalence of insulin resistance as 10%. In addition, the study compared the effect of lopinavir and EFV. Even though both groups developed the same degree of metabolic abnormalities, abnormal fasting glucose was mostly observed in patients taking efavirenz (Steve et al., 2014).

A Danish Nationwide Population-Based Cohort Study found the risk of DM to be higher in HIV infected individuals compared to the comparison cohort both before and after HAART initiation in the period 1996–1999. In the period 1999–2010, the risk of DM in HIV-infected individuals did not differ from that of the comparison cohort though the risk was decreased before HAART initiation (Rasmussen et al., 2012). Overall DM was diagnosed in 3.0% of HIV-infected individuals where 2.7% of the cases were type 2 DM.

A study that compared glucose metabolism and other metabolic changes in antiretroviral naive subjects randomized to nelfinavir or EFV plus dual nucleosides at baseline, weeks 8, 16, 32, 48 and 64 found no significant differences between groups at baseline although modest 10% increase from baseline in IR occurred in the study population as a whole. The use of nelfinavir in combination with a pair of NRTI did not induce insulin resistance more than efavirenz did (Michael et al., 2005).

Another study conducted among HIV infected patients on NNRTI based ART and ART naive patients showed the prevalence of dysglycemia in ART naive patients and patients on ART as 25.7% and 21.9%, respectively (Phyllis et al., 2008). The combined prevalence of dysglycemia did not differ between the ART naive and ART groups. Patients who had only received Efavirenz as the third drug had a higher prevalence of dysglycemia.

A study conducted to assess the risk of dysglycemia and IR in NNRTIs discovered nevirapine containing regimens to have more favorable glucose insulin profile than antiretroviral regimens containing Efavirenz or protease inhibitors (Shahmanesh et al., 2004).

In contrary to Shahmanesh et al, a study conducted among HIV infected south African women who had spent a median of 16 months on first line ART discovered stavudine, efavirenz and nevirapine to be significantly associated with diabetes and dysglycemia at follow-up. This study showed that long term exposure to ART in these women is associated with increases in glucose and insulin levels. The study also showed that plasma glucose values, insulin concentrations and markers of insulin sensitivity and beta cell function have increased significantly from the baseline value during the follow up period. Glucose abnormalities such as diabetes, impaired glucose tolerance and dysglycemia all increased from baseline to follow up but more importantly diabetes increased form baseline of 1% to 7.5% (Zulfa et al., 2015).

Researchers in Cape Town, South Africa also found that EFV was significantly associated with impaired glucose tolerance even after controlling for body mass index and waist circumference (Dave et al., 2011). Participants in that study had all been receiving EFV for at least 6 months at the time of enrolment, suggesting that EFV may accelerate IR.

Another study conducted in Tanzania showed that 24.7% of HIV patients were found to have been diagnosed with diabetes, of whom, Type 2 DM accounted for 92.6% of the cases (Kabati et al., 2010).

A study conducted among HIV patients at Jimma University specialized hospital in Ethiopia showed the prevalence of diabetes mellitus to be 6.4% (Mohammed et al., 2015).

From the above studies, it can be said that the prevalence of IR ranges from 10 – 21 %, whereas the prevalence of DM ranges from 3 – 24.7% among HIV patients receiving HAART. The prevalence of DM is higher in patients than that caused by HIV itself (7%).

1.3.3 Measurement of Insulin resistance

Patients with HIV should be screened for diabetes prior to initiating HAART, and three to six months thereafter. While it is recommended to use fasting blood glucose as a screening tool (Steve et al., 2016), the predominant role of insulin resistance in the development of the illness implies that postprandial glucose values or an oral glucose tolerance test should also be performed as part of screening procedures.

One may follow guidelines for the general population, and screen all HIV patients at diagnosis and at regular intervals, with both fasting and postprandial glucose values (Schambelan et al., 2002, Kalra et al., 2011).

There is no test that can directly detect IR. Instead, a health practitioner will look at an individual's entire clinical picture and may suspect IR if the person has increased glucose, triglyceride and LDL levels and decreased HDL (De wit et al., 2008).

Previous studies showed that increased activities of liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators of hepatocellular injury. Increased activity of these markers was associated with IR, metabolic syndrome, and T2DM (Wannamethee et al., 2005, Nannipieri et al., 2005).

In addition to laboratory parameters, there are mathematical indices that are used to calculate insulin sensitivity where the Hyperinsulinemic Euglycemic Clamp (HEC), was known to be the "gold standard". It requires infusions of insulin and glucose over about 2 hours with measurements of blood glucose every few minutes. However, the time and money consuming nature of the test and impracticability for epidemiological purpose led to the development of simplified approaches in quantification of insulin sensitivity such as the Triglyceride Glucose (TyG) index, among others.

Researchers in Mexico proposed a new formula for estimating IR from triglycerides and glucose, referred to as the triglyceride to glucose (TyG) index based on the product of the natural logarithm between triglycerides (TG) and glucose divided by 2, whose formula is TyG = [(ln fasting triglycerides (mg/dL) x fasting glucose (mg/dL))/2] (Simental-Mendia et al., 2008).

This formula was validated against the Homeostatic Model Assessment Insulin Resistance (HOMA-IR) index in an apparently healthy population. Later, the same group estimated the sensitivity and specificity of this index from 2 populations (healthy subjects and subjects with T2DM) versus the HEC, obtaining a high sensitivity (96.5%) and specificity (85%) using a cutoff point of ≥ 4.68 (Fernando et al., 2010).

TyG index affords an easily and widely available simple laboratory method as a surrogate estimate of insulin sensitivity that could be used repeatedly in large-scale observational and/or

interventional studies. It also offers the advantage of having a standardized method of measuring triglyceride and glucose (Fernando et al., 2010).

A recent study conducted in China has shown an increased risk of T2DM with increasing TyG index for normal-weight people in a Chinese rural cohort. In addition, the study evidenced the usefulness of TyG index in predicting T2DM among normal-weight people (Ming et al., 2017).

1.3.4 Relationship between diabetes, Tuberculosis and anti-Tuberculosis medications

The first report of the association between DM and TB was documented by Avicenna (980-1027 AD) over one thousand years ago. Since that time, the relationship between DM and TB, and the nature of their interaction with regards to co-morbidity are largely suggested by numerous epidemiological studies. More importantly each disease is thought to exacerbate and worsen the outcome for the other (Asfandyar et al., 2012).

1.3.4.1 Diabetes as a risk factor for tuberculosis

Diabetes is an independent risk factor for all lower respiratory tract infections (Workneh et al., 2016 and Asfandyar et al., 2012). A review (Stevenson et al., 2007) of nine studies showed that diabetes increased the risk of TB from 1.5 to 7.8-fold. Even though TB is more strongly associated with other immune deficiency diseases such as HIV, as the number of people with diabetes is much greater than that of patients with other immunocompromised states, it makes DM, a more significant risk factor for TB at the population level.

1.3.4.2 Tuberculosis as a risk factor for diabetes

The relationship between DM and TB is bi-directional. Tuberculosis may lead to development of new diabetes cases (Asfandyar et al., 2012). Studies showed a high prevalence of diabetes, as well as impaired glucose tolerance, in patients with tuberculosis (Jeon et al., 2010).

A study conducted in South Africa found an association between tuberculosis and IR development in newly diagnosed pulmonary tuberculosis patients. Although not significant, IR levels decreased over time, which was, according to the study, indicative of a clinical improvement (Lauren et al., 2017).

1.3.4.3 Anti Tuberculosis medications as risk factors for diabetes

Various conflicting literatures have shown the effect of anti TB medications on the incidence, development or progression of diabetes. Few studies showed isoniazid and rifampicin to have hyperglycemic effects. The effect of rifampicin has also been found to result in transient hyperglycemia soon after treatment commencement due to its strengthening of intestinal glucose absorption (Asfandyar et al., 2012, Girling et al., 1982 and Takasu et al., 1982).

In contrary to this idea, a study conducted in China found that TB patients who do not have DM based on Fasting Blood Glucose (FBG) measurements to not develop DM during anti-TB treatment. Those newly diagnosed with DM on screening in general maintain their DM status with high FBG. Patients with already known DM had their FBG controlled during treatment (Yan et al., 2017).

1.3.5 Measurement of insulin resistance and non-fasting blood tests

Studies have shown that fasting blood tests can be problematic for population - based studies, mainly, because many participants may be unwilling to fast overnight before attending the study: for example, less than half of the participants followed the instruction to fast in the National Health and Nutrition Examination Survey of the US (National Health and Nutrition Examination Survey, 2008).

Some participants may not admit that they have eaten before the test which might lead to measurement variability resulting from the mix of fasting and non-fasting samples and introducing the potential for bias. Fasting also restricts scheduling of assessments because the blood tests are usually taken before breakfast and adds to the time and resources needed for the research because participants may need to be provided with food before undergoing further assessments (National Health and Nutrition Examination Survey, 2008).

Another study suggested the acceptability of using non-fasting bloods to assess insulin resistance in epidemiological studies so that it would avoid a number of difficulties inherent in asking participants to fast overnight and may remove one of the barriers to participation in these studies (Hancox et al., 2011).

In support of the above notion, a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine revealed the of fasting to not be routinely required for determination of a lipid profile (Borge et al., 2016). According to the study, maximal mean changes at 1 - 6 h after habitual meals are not clinically significant for triglycerides, total cholesterol and LDL cholesterol. Furthermore, concentration of HDL cholesterol is not affected by fasting/non-fasting status.

More importantly, the study standardized abnormal lipid profile values for non-fasting samples so that laboratory reports should flag abnormal concentrations as triglycerides ≥175 mg/dL, total cholesterol ≥190 mg/dL, LDL cholesterol ≥115 mg/dL and HDL cholesterol ≤40 mg/dL (Borge et al., 2016).

2. Objectives

2.1 General objective

To determine Efavirenz based ART associated insulin resistance in Ethiopian patients receiving ART alone and in combination with Anti-TB drugs among HIV infected and HIV-TB co infected patients respectively.

2.2 Specific objectives

1. To determine insulin resistance that occurred in HIV only infected patients receiving efavirenz based antiretroviral therapy
2. To determine insulin resistance that occurred in TB-HIV coinfected patients receiving efavirenz based antiretroviral therapy in combination with rifampicin based Anti TB regimens
3. To assess risk factors associated with insulin resistance

3. Methods and materials

3.1 Study setting and Design

This study was conducted as one of the sub studies designed under the umbrella of the broad clinical research named the HIV-TB pharmagene Study in Ethiopia (Habtewold et al., 2016). For the pharmagene study, patients were recruited from different study sites within Addis Ababa, the capital city of Ethiopia, including Tikur Anbessa Specialized Hospital (TASH) and 4 other health centers. It was conducted between June 2007 and June 2011 at HIV and TB clinics in Addis Ababa, Ethiopia.

The blood sample of patients whose blood was collected between November 2010 and February 2011 were selected. The laboratory test was performed at Hawassa University Specialized Hospital analytical chemistry laboratory, between February 2 and 20, 2019.

A study design employed was a prospective, comparative, parallel assignment two-arm cohort study. A total of 60 blood samples of patients 18 years and above who received efavirenz based ART alone and in combination with anti-TB drugs, 30 in each arm were included in the study.

3.2 Sample size calculation

The sample size was calculated on the basis of the following assumptions: use of the Unmatched Cohort and Cross-Sectional Studies formula with a 95% confidence level; power of 80%; Ratio of controls to cases 1; percent outcome in unexposed group 15.5% (*taking the average IR prevalence based on the literatures*); percent outcome in exposed group 50% (*no previous studies are available*). The final sample size, as calculated using Epi-Info Version.7.2.2statistical software for medical research studies (Atlanta, Georgia, USA), was 60.

3.3 Eligibility criteria

3.3.1 Inclusion criteria

The inclusion criteria for this study were blood samples of HIV positive men and non-pregnant women age ≥18 years, and CD4 count less than 200 cells per mm^3 at baseline. Blood sample of patients with a CD4 less than 200 cells/mm^3 was selected because various studies indicated an association between IR and a CD4 count below 200 cells/mm^3.

3.3.2 Exclusion criteria

Exclusion criteria were histories suggestive of liver diseases and previous history of DM. Patients with liver diseases were excluded because of possible stress hyperglycemia which may act as a cofounder.

3.4 Sampling technique

The study participants were grouped in two arms. Arm 1 included blood samples obtained from ART naive HIV infected individuals without active TB. Arm 2 included blood samples obtained from treatment naive patients with active TB – HIV co infection who were then put on ART and Anti TB medications.

For HIV treatment group, samples at baseline, week 4 and week 16 were selected; for TB – HIV co-treatment group, samples at baseline, weeks 8 and week 20 were selected. In TB – HIV cotreatment group, ART was initiated 4 weeks after the initiation of anti TB drugs. Therefore, the baseline (time of anti TB initiation) was expressed as week (-4). The 8^{th} week on anti TB coincides with 4 weeks on ART. Similarly, 20^{th} week on anti TB coincides with 16 weeks of stay on ART. Week 8 and 20 in TB – HIV cotreatment group coincides with week 4 and 16 of HIV only treatment group. In other words, the time frames at week 4 and 16 between the two treatment groups i.e. HIV only treatment group and TB-HIV cotreatment group, was similar in terms of ART duration.

Representative blood samples of study participants were selected using a convenience sampling technique.

The flow chart is shown in figure 2.

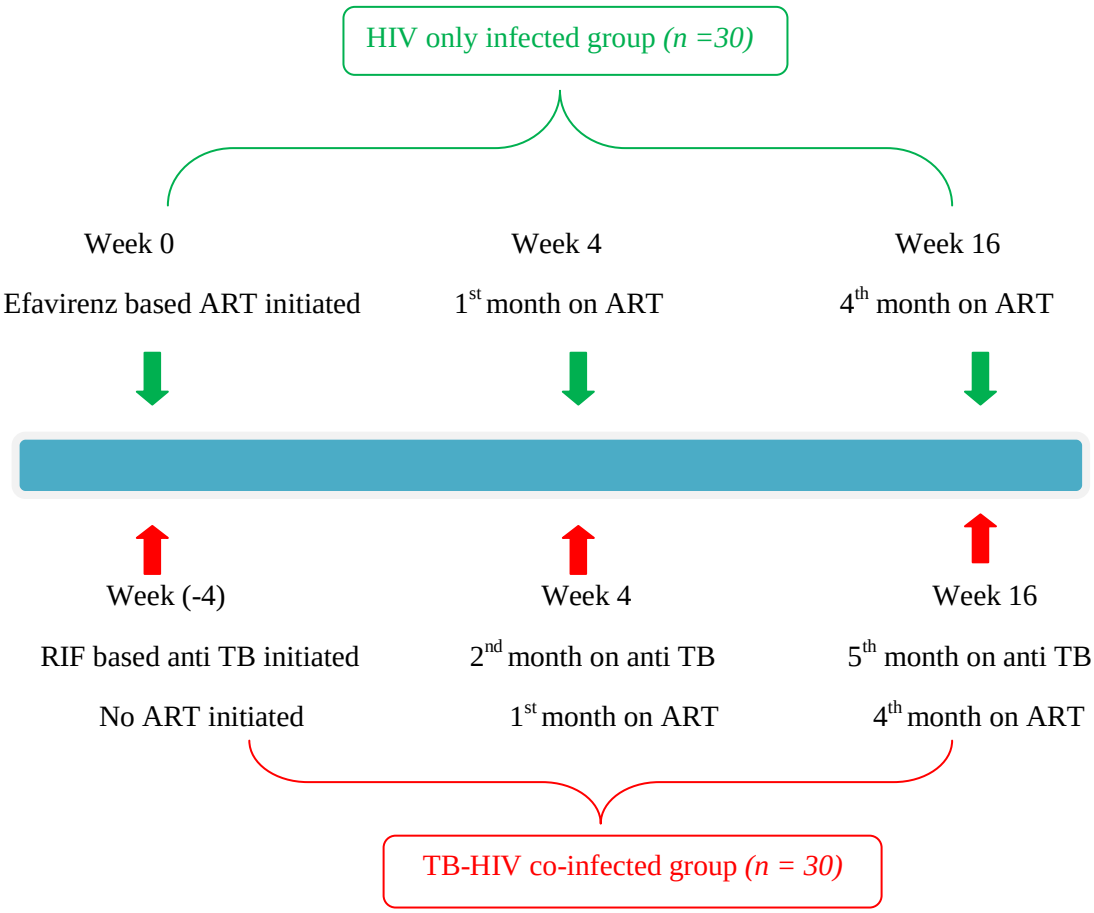

Figure 2 – Study design stratified by treatment group in HIV only treatment group receiving EFV based ART and TB-HIV co-treatment group receiving efavirenz based ART in combination with rifampicin based Anti tuberculosis medications (Adopted from Habtewold et al., 2016 with modification).

3.5 Data collection

Specimens for previous studies were collected in iced, heparinized tubes. The plasma samples were separated from blood cells with ultracentrifugation for 10 minutes at 10,000 rpm. Plasma was aliquoted into cryotubes and stored at –80°C with aseptic precautions until shipment to analytical chemistry laboratory, Hawassa University Specialized Hospital for analysis. To determine insulin resistance: plasma glucose, liver enzymes (ALT, AST and ALP) and lipid profile tests (Cholesterol, HDL, LDL and TG) were performed.

Patients' medical records were reviewed to collect demographic data and identify risk factors for developing insulin resistance (e.g. age, sex, BMI, medication history and hepatitis C virus status). Data on antiretroviral regimens, baseline CD4 and viral load counts were also collected.

3.6 Ethical consideration

For the Pharmagene study, all patients provided written informed consent to participate. For the current study, blood sample of these study participants stored in deep freezer at -80 was used for determination of insulin resistance. The Pharmagene study protocol received approvals from the Institutional Review Board of the College of Health Sciences, Addis Ababa University and National Research Ethics Review Committee, and the study was conducted as per International Conference for Harmonization, Good Clinical Practice guidelines.

Similarly, the present study obtained an approval from the institutional review board of the College of Health Sciences, Addis Ababa University (Document review code – AAUMF 01-008, Protocol number: 023/18/Pharma, Date – November 5, 2018) prior to its initiation.

In order to keep confidentiality of patient information, each patient sample was coded and only patient initials were used.

3.7 Data processing and statistical analysis

Data entry, processing and analysis were performed using Statistical Package for Social Science (SPSS) version 23.0 (SPSS Inc., Chicago, Illinois, USA).

An independent two samples *t* test was used to compare the rate of insulin resistance, based on TyG index, between the two treatment groups stratified by their exposure status i.e. HIV infected and TB-HIV co-infected. In case where Levene's test for variance was greater than 0.05, equal variance was assumed, otherwise unequal variance was assumed.

Comparison of IR, plasma glucose, liver enzymes and lipid tests between baseline, week 4 and 16 within subjects was analyzed using repeated measures ANOVA. By categorizing the TyG index values in two categories based on a cut point of ≥ 4.68 (Simetal-Mendia et al., 2008), as non-insulin resistant and insulin resistant, categorical independent variables were analyzed for association using a chi square test. Significant value from Pearson's chi square, Fisher's Exact Test and Likelihood Ratio were obtained.

Few continuous independent variables including laboratory parameters were analyzed by binary logistic regression analysis using stepwise backward elimination method.

Model fitness was assessed using the Hosmer and Lemeshows test of goodness fit. In addition, by taking the raw TyG index data, association between insulin resistance and stage of HIV and type of HAART regimen was analyzed using a one – way ANOVA where a Bonferroni corrected post hoc analysis was performed.

A p value of < 0.05 was considered statistically significant. Data were expressed as mean ± SD for continuous variables, and as frequencies for categorical variables.

3.8 Study variables

3.8.1 Dependent variable

Insulin resistance

3.8.2 Independent variables

Demographic variables

- Gender
- Age
- Baseline BMI

Clinical variables

- Baseline CD4
- Type of HAART
- HIV stage

Laboratory variables

- Plasma glucose
- Liver enzymes (ALT, AST and ALP)
- Lipid profile tests (Cholesterol, TG, LDL, and HDL)

4. Results

4.1 Demographic characteristics and baseline laboratory parameters

A total of 60 blood samples of study participants were analyzed in this study. Samples were grouped into two treatment arms: blood samples of HIV patients without active TB infection (Arm 1) and TB HIV co-infected patients (Arm 2). Arm 1 received EFV-based ART (n = 30) consisting of lamivudine (3TC) with either zidovudine (AZT) or stavudine (d4T) or tenofovir (TDF) and Arm 2 received concomitant EFV-based ART with RIF-based anti-TB therapy (n = 30) with isoniazid + pyrazinamide + ethambutol. The demographic characteristics and baseline biochemical laboratory parameters of the study populations is presented in Table 1.

Pretreatment laboratory tests including CD4 cell count, HIV RNA determination, hepatitis B surface antigen, anti-hepatitis C antibody, renal function tests, liver function tests were performed. CD4 cell count and plasma HIV -1 RNA was determined before the initiation of ART. Additionally, renal function test employing creatinine and blood urea nitrogen was determined.

Following this, blood sample of patients whose baseline laboratory parameters were found to be in the normal range are tested and followed for plasma glucose, liver enzymes (ALT, AST and ALP), and lipid profile tests (total cholesterol, triglycerides, LDL and HDL) for three different phases. For HIV only infected group, the tests were conducted at baseline, week 4 and week 16 of ART initiation; whereas for the TB-HIV co-infected group, the tests were conducted at baseline, week 8 and week 20 of anti TB initiation. In TB–HIV co-infected patents, RIF-based anti-TB therapy was initiated 4 weeks prior to starting ART. In both treatment groups, the laboratory tests were determined in parallel at the 4^{th} and 16^{th} week of post-ART initiation (which corresponds to the intensive phase and the continuation phase of anti-TB therapy in TB–HIV co-infected patients, respectively).

Table 1 – Baseline demographic, clinical and laboratory characteristic of study populations

Variables	Treatment Group: HIV Treatment (n = 30)	Treatment Group: TB HIV Cotreatment (n = 30)	*p* - value
I. Continuous variables	Mean ± SD		
Age (years)	35.60 ± 9.66	36.53 ± 11.25	0.732
BMI[a](kg/m^2)	20.43 ± 2.85	19.30 ± 3.16	0.153
Aspartate aminotransferase (AST, U/L)	45.93 ± 45.48	46.03 ± 22.44	0.991
Alanine aminotransferase (ALT, U/L)	36.90 ± 20.03	32.33 ± 15.61	0.329
Alkaline phosphatase (ALP, U/L)	94.60 ± 31.62	100.83 ± 46.50	0.546
Total Bilirubin (mg/dL)	0.59 ± 0.40	0.58 ± 0.45	0.829
Direct Bilirubin (mg/dL)	N/A[b]	0.18 ± 0.16	-
Albumin (g/dL)	N/A	4.05 ± 3.67	-
Creatinine (mg/dL)	N/A	0.97 ± 0.28	-
Blood urea nitrogen (mg/dL)	N/A	18.56 ± 5.52	-
CD4[c] (cells /mm^3)	99.87 ± 54.78	84.93 ± 59.55	0.316
HIV RNA[d] (log10 copies/mL)	5.45 ± 5.54	5.48 ± 5.58	0.812
Random plasma glucose (mg/dL)	90.00 ± 25.55	93.73 ± 22.11	0.547
Total cholesterol (mg/dL)	126.87 ± 32.46	129.24 ± 29.70	0.769
High density lipoprotein (HDL, mg/dL)	29.26 ± 5.36	29.84 ± 3.96	0.636
Low density lipoprotein (LDL, mg/dL)	67.29 ± 9.57	71.94 ± 14.26	0.143
Triglycerides (TG, mg/dL)	138.52 ± 15.31	128.39 ± 27.75	0.110
TyG[e] index	4.71 ± 0.16	4.69 ± 0.15	0.377
II. Categorical variables			
	Frequency in each subcategory		
Sex (male, female)	7, 23	14, 16	
WHO HIV stage			
Stage 1	1	-	
Stage 2	4	1	
Stage 3	10	21	
Stage 4	15	8	
ART[f] (zidovudine, stavudine, tenofovir)	18,11,1	4, 13, 13	
Hepatitis B virus status (Yes, No)	0, 30	0, 30	
Hepatitis C virus status (Yes, No)	0, 30	0, 30	

[a]BMI, Body Mass Index

[b]N/A, Not Available

[c]CD4, Cluster of Differentiation 4

[d]HIV RNA, Ribonucleic acid of human immunodeficiency virus

[e]TYG index, Triglyceride to Glucose index

[f]ART, antiretroviral therapy plus (lamivudine + efavirenz).

There were no statistical differences in baseline demographic, clinical and laboratory characteristics between both treatment groups. More importantly, the observation that none of the study participants were found to be seropositive for Hepatitis C Virus (HCV) at baseline helped to rule out one of the independent risk factors in the development of IR in HIV infected patients..

4.2 Determination of insulin resistance by calculated TyG insulin sensitivity index

The current study used the Triglyceride to Glucose index (TyG index), the latest and cost-effective surrogate marker, as a predictor and determinant of insulin resistance. The TyG index was calculated using the formula provided by Simental-Mendia *et al*. and a value of ≥ 4.68 was assumed to define IR. Accordingly, the TyG index in consecutive weeks was calculated within subjects and between groups. For the present study, random plasma glucose and random triglyceride data was used. The rationale for using this cut point is because of the absence of a cut point for non fasting blood and more importantly because the glucose and triglyceride values fulfilled the fasting standards.

At baseline, the mean TyG index (Mean ± SD = 4.71 ± 0.16); $p = 0.377$ was higher in the HIV only treatment group than the TB-HIV co-treatment group (Mean ± SD = 4.69 ± 0.15). However, it was similar in the following weeks 4 (Mean ± SD = 4.74 ± 0.13 and 4.74 ± 0.14); $p = 0.912$ and 16 (Mean ± SD = 4.78 ± 0.13 and 4.78 ± 0.11); $p = 0.993$. (Figure 3)

Despite the mean difference between groups, an independent two samples *t* test, assuming equal variances Levene's test for equality of variances $p > 0.05$, indicated that the treatment groups did not differ significantly, and the mean TyG index for the HIV only treatment group was not significantly different from the TB-HIV co-treatment group at baseline, weeks 4 and 16.

A within subject analysis showed a significantly different mean TyG index between baseline and week 16 in HIV only treatment group (mean difference = - 0.07; 95% CI for the mean difference = - 0.214 to - 0.033); $p < 0.01$. In the TB-HIV co-treatment group, however, a significant mean difference was observed between baseline and week 4 (mean difference = - 0.05; 95% CI for mean difference = - 0.159 to - 0.015); $p = 0.013$ and between baseline and week 16 (mean difference = - 0.09; 95% CI for mean difference = - 0.250 to - 0.070); $p < 0.01$.

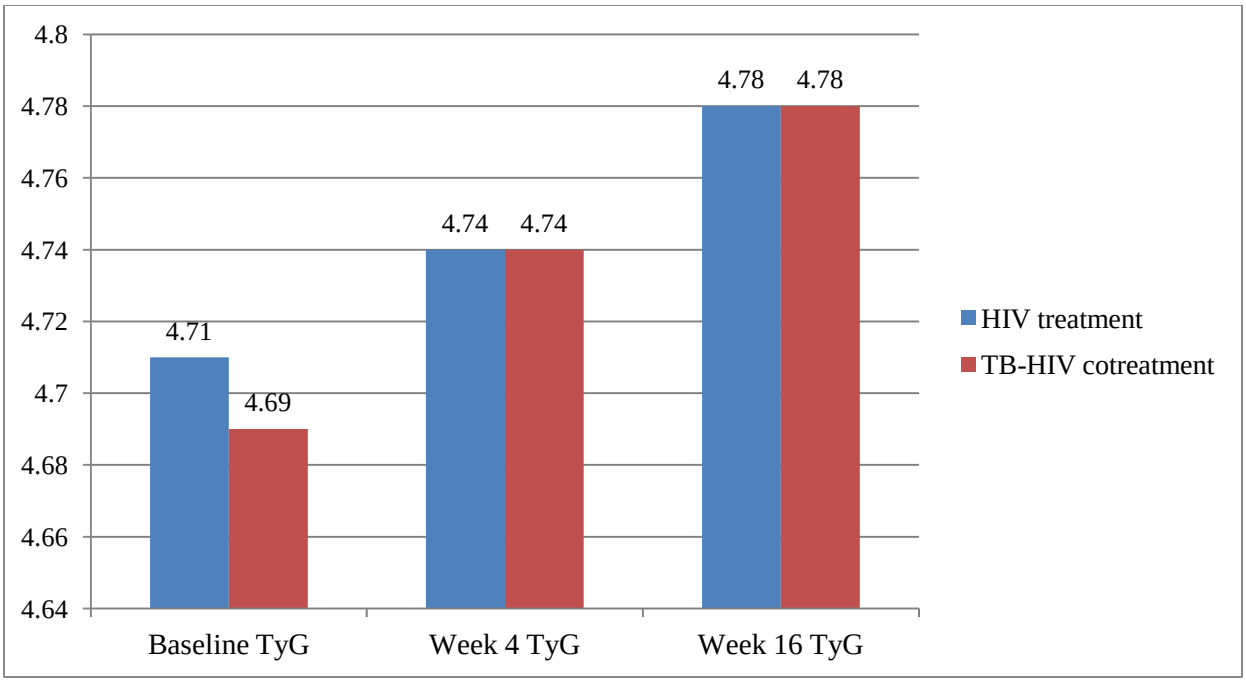

Figure 3 – Comparison of mean TyG index between HIV patients treated with efavirenz-based antiretroviral therapy only (efavirenz) or HIV–tuberculosis co-infected patients co-treated with rifampicin based anti-tuberculosis therapy (efavirenz + rifampicin). TyG index was measured at baseline, 4th and 16th weeks of antiretroviral therapy.

4.3 Effect of rifampicin-based anti-Tuberculosis treatment on insulin resistance

It was observed that in patients who received Anti TB drugs together with EFV based ART; a significant insulin resistance was observed as measured by TyG index. The finding shows that the incidence is somehow similar to the HIV only treatment group.

The mean TyG index showed a significant elevation across the study period. At baseline i.e. before the initiation of ART, an IR was observed in such study cohorts.

Despite the fact that IR was observed at baseline; the mean baseline TyG index was lower than the HIV only treatment group. After the initiation of ART, however, the TyG index became similar to the HIV treatment group. (Figure 4)

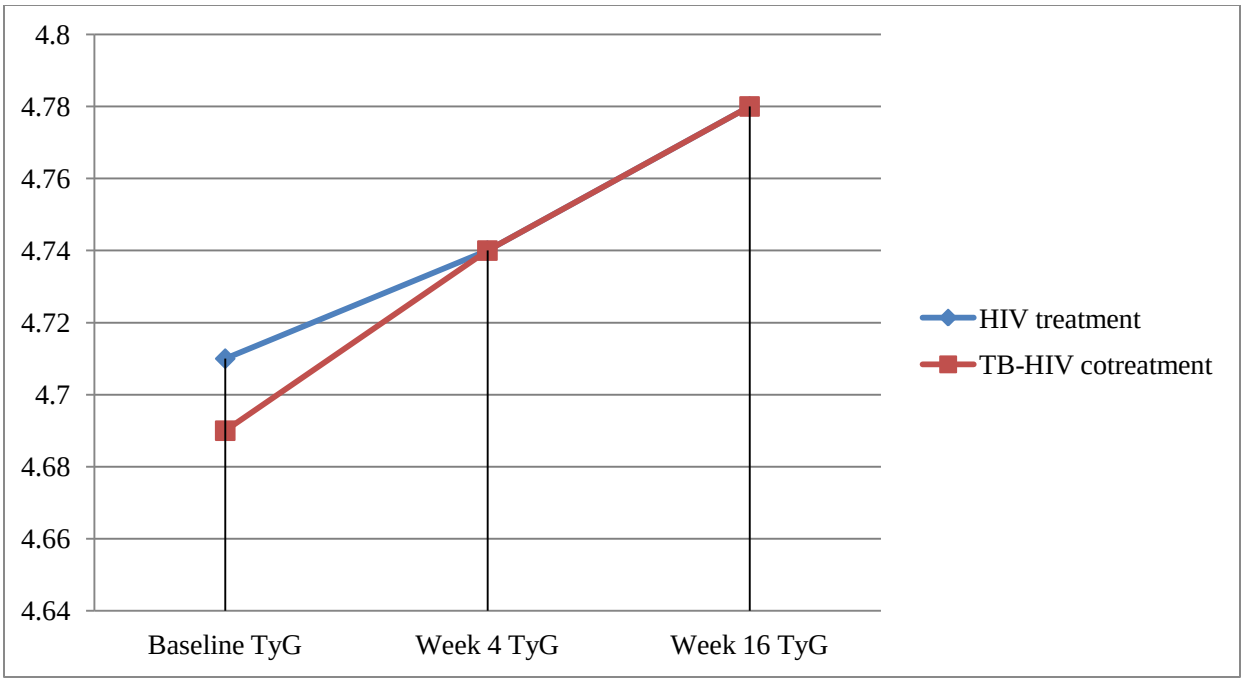

Figure 4 – Mean TyG index in HIV–tuberculosis co-infected patients co-treated with rifampicin based anti-tuberculosis therapy (efavirenz + rifampicin) in comparison with the HIV only treatment group.

4.4 Association between insulin resistance and risk factors

A chi square test was performed to look for association between IR and age, gender, BMI, HAART type and HIV stage TyG index was categorized into two based on a cut off value as non-insulin resistant (< 4.68) and insulin resistant (≥ 4.68). Similarly, variables such as baseline CD4 count, baseline viral load, plasma glucose level, liver enzymes and lipid profile tests were analyzed using a binary logistic regression. Association between HIV stage and type of HAART was also analyzed using a one-way ANOVA.

4.4.1 Association with Gender

A higher mean TyG index values was observed in male study participants of both treatment groups. However, a chi square test has shown that there was no statistically significant relationship between gender and TyG index at baseline (p = 066 and 0.96), week 4 (p = 0.84 and 0.51) and week 16 (p = 0.41 and 0.27) in HIV only treatment and TB-HIV cotreatment groups respectively.

4.4.2 Association with Age

Age was categorized into two: as below and above 40. In the current study, a higher mean TyG index was observed in patients aged above 40 between both treatment groups at baseline, week 4 and week 16. However, age was not associated with TyG index at baseline (p = 0.53 and 0.79), week 4 (p = 0.71 and 0.48) and week 16 (p = 0.44 and 0.47) in HIV only treatment and TB HIV cotreatment groups respectively.

4.4.3 Association with Body Mass Index

Body mass index (BMI) of participants was categorized into two as below and above 18.5kg/m^2. The findings have shown that a higher mean TyG index was observed in patients whose BMI was below 18.5kg/m^2. BMI was not associated with TyG index at baseline (p = 0.29 and 0.88) week 4 (p = 0.30 and 0.06) and 16 (p = 0.41 and 0.40) in HIV only treatment and TB-HIV co-treatment groups.

4.4.4 Association with HIV stage

In the present study, the highest mean TyG index was observed in stage 3 and 4 patients of both treatment groups. However, a chi square test has shown that HIV stage was not associated with TyG index at baseline (p = 0.081), week 4 (p = 0.380) and week 16 (p = 0.60) in HIV only treatment group. Similarly, in TB-HIV co-treatment groups, there was no statistically significant relation between HIV stage and TyG index at baseline (p = 0.480), week 4 (p = 0.871) and week16 (p = 0.638).

By taking the raw (uncategorized) TyG index data, a one-way ANOVA following Bonferroni adjusted post hoc analysis has indicated that there was a statistically significant difference between groups at weeks 4 and 16 stratified by HIV stage. In HIV only treatment group, a higher mean TyG was found in HIV stage 4 patients at week4 (p = 0.034) and week 16 (p = 0.049). In TB-HIV co-treatment groups, a higher mean TyG index was found in stage 4 HIV (p = 0.019) at week 16.

4.4.5 Association with type of HAART regimen

According to a chi square test, no association between the type of HAART and TyG index was found at baseline ($p = 0.358$), week 4 ($p = 0.826$) and week 16 ($p = 0.343$) in HIV only treatment group. The same was true in TB-HIV co-treatment groups.

Similarly, a one-way ANOVA indicated that there was no statistically significant mean difference in TyG index across the different ART regimens even though higher mean TyG index was observed in patients taking stavudine. More importantly, the Bonferroni-corrected post hoc analysis revealed no significant mean difference in TyG index between the HIV only infected group and the TB-HIV coinfected group, regardless of the presence or absence of other HAART regimen components i.e. zidovudine, stavudine and tenofovir in the present study.

Summary of mean Triglyceride to glucose index in categorical variables is presented in table 2.

Table 2 – Comparison of mean triglyceride to glucose (TyG) index between patients receiving efavirenz-based antiretroviral therapy alone or with rifampicin based anti-tuberculosis therapy at baseline, week 4 and week 16 stratified by gender, age, BMI, HIV stage and type of HAART regimen.

	HIV only treatment group Mean ± SD			TB-HIV co treatment group Mean ± SD		
	Baseline TyG	Week 4 TyG	Week 16 TyG	Baseline TyG	Week 4 TyG	Week 16 TyG
Gender						
Male	4.78 ± 0.12	4.84 ± 0.15	4.90 ± 0.16	4.72 ± 0.16	4.81 ± 0.13	4.90 ± 0.11
Female	4.74 ± 0.18	4.79 ± 0.12	4.87 ± 0.13	4.71 ± 0.14	4.78 ± 0.14	4.87 ± 0.11
Age						
< 40	4.72 ± 0.17	4.75 ± 0.11	4.81 ± 0.12	4.70 ± 0.11	4.75 ± 0.15	4.82 ± 0.10
≥ 40	4.75 ± 0.10	4.79 ± 0.16	4.84 ± 0.16	4.73 ± 0.21	4.80 ± 0.12	4.83 ± 0.12
BMI						
< 18.5 kg/m^2	4.76 ± 0.07	4.81 ± 0.06	4.83 ± 0.12	4.74 ± 0.15	4.83 ± 0.11	4.84 ± 0.10
≥ 18.5 kg/m^2	4.73 ± 0.18	4.77 ± 0.14	4.82 ± 0.13	4.68 ± 0.14	4.76 ± 0.14	4.80 ± 0.11
HIV stage						
Stage 1	4.18 ± 0.09	4.70 ± 0.10	4.71 ± 0.14	N/A	N/A	N/A
Stage 2	4.78 ± 0.11	4.79 ± 0.02	4.81 ± 0.12	4.68 ± 0.12	4.70 ± 0.08	4.84 ± 0.11
Stage 3	4.79 ± 0.08	4.83 ± 0.12	4.85 ± 0.12	4.71 ± 0.14	4.79 ± 0.13	4.90 ± 0.10
Stage 4	4.79 ± 0.21	4.86 ± 0.12	4.90 ± 0.12	4.78 ± 0.19	4.84 ± 0.17	4.96 ± 0.08
HAART regimen						
AZT/3TC/EFV	4.73 ± 0.20	4.81 ± 0.12	4.86 ± 0.14	4.70 ± 0.08	4.78 ± 0.23	4.82 ± 0.14
d4T/3TC/EFV	4.79 ± 0.09	4.81 ± 0.15	4.89 ± 0.13	4.71 ± 0.16	4.87 ± 0.11	4.89 ± 0.10
TDF/3TC/EFV	4.74 ± 0.13	4.77 ± 0.14	4.82 ± 0.10	4.72 ± 0.16	4.80 ± 0.08	4.88 ± 0.11

Note – No significant association between gender, age, baseline BMI, HIV stage and type of HAART regimen and insulin resistance was observed between patients receiving efavirenz-based antiretroviral therapy alone or with rifampicin-based anti-tuberculosis therapy at baseline, week 4 and week 16. However, higher mean TyG index was observed in male, age ≥ 40, BMI < 18.5kg/m^2, HIV stage 4 and patients receiving stavudine based HAART regimens. This higher mean value was similar in both treatment groups. AZT – zidovudine; BMI – Body Mass Index; d4T – stavudine; HAART – Highly Active Antiretroviral Therapy; N/A – Not Available; TDF – Tenofovir Disoproxil Fumarate; 3TC – lamivudine; TyG – Triglyceride to Glucose index.

4.4.6 Association with baseline CD4

The present study analyzed the baseline CD4 to look for an association with TyG index at the three-time frames. Accordingly, the analysis indicated that a CD4 below 200cells/mm^3 was not a predictor of elevated TyG index and IR.

Table 3 – Binary logistic regression analysis of baseline CD4 between patients receiving efavirenz-based antiretroviral therapy alone or with rifampicin-based anti-tuberculosis therapy at baseline, week 4 and week 16

	HIV treatment		**TB-HIV co-treatment**	
Parameter	**OR (95% CI)**	***p* value**	**OR (95% CI)**	***p* value**
Baseline TyG	- 0.218 (0.576, 1.122)	0.200	- 0.063 (0.743, 1.18)	0.602
Week 4 TyG	- 0.130 (0.623, 1.237)	0.457	- 0.405 (0.433, 1.028)	0.066
Week 16 TyG	0.210 (0.679, 2.242)	0.491	0.121 (0.692, 2.154)	0.713

OR, Odds Ratio; TyG, Triglyceride to Glucose index

4.5 Association with laboratory parameters

4.5.1 Association with plasma glucose level

A within subject analysis showed no significantly different mean plasma glucose at baseline, week 4 and week 16 in HIV only treatment group and TB-HIV co-treatment group. The mean random plasma glucose was higher for patients taking RIF based anti TB medications in combination with EFV based ART at baseline, week 4 and 16. However, an independent two samples *t* test indicated no statistically significant difference in the mean plasma glucose between groups at baseline, weeks 4 and 16. Similarly, no significant association between plasma glucose and TyG index was observed following a binary logistic regression analysis.

Table 4 – Comparison of mean plasma glucose between patients receiving efavirenz based antiretroviral therapy alone or with rifampicin-based anti-tuberculosis therapy at baseline, week 4 and week 16

Plasma Glucose	HIV treatment (Mean ± SD)	TB-HIV co-treatment (Mean ± SD)	95% CI for mean difference	*p* value
Baseline	90.00 ± 25.55	93.73 ± 22.11	(-16.08, 8.61)	0.547
Week 4	94.33 ± 28.86	101.67 ± 24.97	(- 21.28, 6.62)	0.297
Week 16	97.37 ± 31.42	104.93 ± 23.73	(-15.96, 12.82)	0.828

4.5.2 Association with liver enzymes

In the present study, the mean plasma ALT was higher in HIV only treatment group than the TB-HIV co-treatment group at baseline, week 4 and week 16. However, the mean plasma AST and ALP values were higher in TB-HIV co-treatment groups. Despite the mean differences in ALT, AST and ALP between the treatment groups, elevations in the mean values were observed at the three-time frames in both treatment groups.

An independent 2 samples *t* test indicated that the mean ALT and AST value between the groups was not significantly different. However, the test, not assuming equal variances Levene's test for equality of variances $p < 0.05$, indicated that the mean ALP value was higher in TB-HIV co-treatment group at week 4 (Mean ± SD = 127.13 ± 67.78) and week 16 (Mean ± SD = 158.97 ± 59.30) than the HIV only treatment group(Mean ± SD = 98.27 ± 33.03; mean difference = -28.86; 95% CI for mean difference = -56.65 to -1.08); $p = 0.042$ and 16 (Mean ± SD = 120.37 ± 35.38; mean difference = - 38.60; 95% CI for mean difference = - 63.96 to -13.24); $p < 0.01$.

Within subject analysis showed that there was no significant difference in mean plasma ALT in HIV only treatment and TB-HIV co-treatment group at the three-time frames. Accordingly, no significant difference in the mean plasma AST was observed in HIV only treatment group. However, a significant mean difference in plasma AST was observed between baseline and week 16 ($p = 0.043$) in TB-HIV co-treatment group.

In addition, the analysis showed that there was a significant difference in mean plasma ALP in HIV treatment group between baseline and week 16 ($p = 0.01$) and between week 4 and week 16

(p = 0.018). However, in TB-HIV co-treatment group a significant mean difference was observed between baseline and week 16 (p < 0.01).

A binary logistic regression analysis indicated that there was no association between insulin resistance and abnormalities in liver enzymes. In other words, elevations in liver enzymes were not predictors of insulin resistance in HIV only treatment and TB-HIV co-treatment groups.

Table 5 – Comparison of mean of ALT, AST and ALP between patients receiving efavirenz based antiretroviral therapy alone or with rifampicin based anti-tuberculosis therapy at baseline, week 4 and week 16

LFTs	HIV treatment (Mean ± SD)	TB - HIV cotreatment (Mean ± SD)	95% CI for Mean difference	*p* value
ALT				
Baseline	36.90 ± 20.03	32.33 ± 15.61	(- 4.71 to 13.84)	0.329
Week 4	38.57 ± 21.99	33.87 ± 15.84	(- 5.20 to 14.60)	0.346
Week 16	44.00 ± 29.71	37.97 ± 28.89	(- 9.11 to 21.18)	0.429
AST				
Baseline	45.93 ± 45.48	46.03 ± 22.44	(-18.63 to 18.43)	0.991
Week 4	46.47 ± 34.39	48.87 ± 15.84	(- 1.36 to 26.56)	0.074
Week 16	48.50 ± 35.89	59.73 ± 36.66	(- 29.98 to 7.51)	0.235
ALP				
Baseline	94.60 ± 31.62	100.83 ± 46.50	(- 26.78 to 14.32)	0.546
Week 4	98.27 ± 33.03	127.13 ± 67.78	(- 56.65 to -1.08)	0.042*
Week 16	120.37 ± 35.38	158.97 ± 59.30	(- 63.96 to -13.24)	< 0.01*

ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALP - Alkaline phosphatase; LFTs - Liver function tests; * - Significant at < 0.05

4.5.3 Association with lipid profile

The lipid panel test was also performed to look for association with IR. Accordingly, highest mean values of total cholesterol and triglyceride and lowest mean values of HDL were observed

in the HIV only treatment group; while highest mean values of LDL values were observed in the TB-HIV co-treatment group.

A repeated measure ANOVA indicated that mean plasma cholesterol, mean plasma triglyceride, mean plasma LDL level and mean plasma HDL level differences were observed within subjects in both treatment groups and were significantly different. In all the lipid panel tests, a significant difference was observed between baseline and week 4 ($p < 0.01$), between week 4 and week 16 ($p < 0.01$) and between baseline and week 16 ($p < 0.01$).

Similarly, an independent two samples *t* test indicated that the mean plasma cholesterol at week 4 was higher in HIV only treatment group (Mean ± SD = 165.45 ± 20.10) than the TB-HIV co-treatment group (Mean ± SD = 146.65 ± 31.21; mean difference = 18.80; 95% CI for mean difference = 5.18 to 32.42); $p < 0.01$. In addition, the mean plasma triglyceride at week 4 (Mean ± SD = 140.88 ± 13.39) and week 16 (Mean ± SD = 147.98 ± 11.44) were higher in HIV only treatment group than the TB-HIV co-treatment group (Mean ± SD = 130.91 ± 24.29; mean difference = 9.97; 95% CI for mean difference = 7.47 to 23.71); $p = 0.014$ and (Mean ± SD = 137.90 ± 12.84; mean difference = 10.08; 95% CI for mean difference = 2.76 to 18.16); $p = p < 0.01$ respectively. In contrast, there was no significant difference in mean LDL and HDL between treatment groups. (Table 6)

Table 6 – Comparison of mean cholesterol, TG, LDL and HDL between patients receiving efavirenz based antiretroviral therapy alone or with RIF based anti-tuberculosis therapy at baseline, week 4 and week 16

Lipid panel	HIV treatment (Mean ± SD)	TB-HIV cotreatment (Mean ± SD)	95% CI for mean difference	*p* value
Cholesterol				
Baseline	126.86 ± 32.46	129.23 ± 29.70	(- 18.45 to 13.71)	0.769
Week 4	165.45 ± 20.10	146.65 ± 31.21	(5.23 to 32.37)	< 0.01*
Week 16	184.73 ± 15.53	175.78 ± 21.58	(-0.77 to 18.66)	0.07
Triglyceride				
Baseline	138.52 ± 15.31	128.39 ± 27.75	(- 1.20 to 11.37)	0.11
Week 4	140.88 ± 13.39	130.91 ± 24.29	(7.47 to 23.71)	0.014*
Week 16	147.98 ± 11.44	137.90 ± 12.84	(2.76 to 18.16)	< 0.01*
LDL				
Baseline	67.29 ± 9.57	71.94 ± 14.26	(- 10.95 to 1.64)	0.14
Week 4	89.16 ± 13.43	88.69 ± 15.06	(- 6.91 to 7.84)	0.90
Week 16	107.24 ± 9.71	104.48 ± 14.36	(- 3.59 to 9.11)	0.38
HDL				
Baseline	29.26 ± 5.36	29.84 ± 3.96	(- 3.01 to 1.85)	0.63
Week 4	36.95 ± 4.58	38.62 ± 5.04	(- 4.16 to 0.81)	0.18
Week 16	41.28 ± 4.26	42.37 ± 3.46	(- 3.09 to 0.91)	0.28

HDL – High Density Lipoprotein; LDL – Low Density Lipoprotein; * - significant at <0.05

A binary logistic regression analysis indicated that none of the lipid profile tests were associated with TyG index at baseline, week4 and week16 in HIV only treatment groups. In TB-HIV co-infected groups, however, baseline plasma triglyceride was associated with TyG index and insulin resistance (OR, 95% CI = 1.170; $p < 0.01$). Therefore, it can be said that for a unit increase in plasma triglyceride level, the log odds that TyG index is elevated increases by 0.157. (Table 7)

Table 7 – Binary logistic regression analysis of baseline lipid profile tests in TB – HIV coinfected patients receiving efavirenz based antiretroviral therapy in combination with rifampicin based anti-tuberculosis therapy.

	B	**P**	**OR**	**95% C.I. for EXP(B)**	
				Lower	Upper
Baseline cholesterol	- 0.066	0.06	.936	0.873	1.003
Baseline triglyceride	0.157	< 0.01*	1.170	1.049	1.305
Baseline LDL	- 0.141	0.07	.869	0.745	1.014
Baseline HDL	- 0.098	0.56	.906	0.648	1.268

B – Regression coefficient; OR – Odds Ratio; p – significance; * - significant at $p < 0.05$

5. Discussion

The present comparative study aimed to determine insulin resistance in HIV only infected and TB – HIV co-infected patients who were naive to HAART regimens and to look for trends of insulin resistance as a predictor of T2DM. This procedure i.e. performing the analysis on HAART naive patients is also suggested by American diabetes association (American Diabetes Association, 2018) and various studies. For instance, one study recommends patients with HIV to be screened for diabetes prior to initiating HAART, and three to six months after initiating HAART (Diana et al., 2007). Furthermore, the present study tried to screen patients for diabetes three to six months after initiation of HAART. This is also in line with suggestions provided by American diabetes association and study of Diana and her colleagues.

In addition, the present study tried to look for the trend of hyperglycemia after initiation of HAART. Similarly, a study identified three subgroups of patients with DM and HIV. These were patients with preexisting diabetes who contract HIV, patients diagnosed to have diabetes at the onset of HIV infection and patients who develop hyperglycemia after starting HAART (Kalra et al., 2011). Therefore, the present study screened patients who had the potential to develop hyperglycemia after starting HAART which might by ART.

In the current study, IR was present between the treatment groups indicated at baseline. This indicates that insulin resistance might be related to HIV infection itself and could be exacerbated with the initiation of highly active antiretroviral treatment. The role of HIV infection itself was witnessed with few studies similar to the present study (Brown et al., 2005 and Dube, 2000). The mechanism by which HIV led to IR was due to the direct effects of the HIV virus on pancreatic betta cell function and insulin secretion (Calmy et al., 2007). In addition, the observation of IR in the present study participants before the initiation of HAART may also be correlated with innate immune system activation. Similar to this, a study conducted by Marcelo *et al.*, indicated that immune activation resulted in chronic inflammation that varies in severity, and was observed in untreated HIV patients (Marcelo et al., 2018). In contrast to this, one study indicated an increasing trend of IR which was normal at baseline (Zulfa et al., 2015). The reason for this difference might be related to the index used. In the later study, HOMA-IR rather than TyG index was used as a surrogate marker.

The baseline TyG value is especially critical because the baseline observation of IR in the present study participants may signify the potential of IR to develop into diabetes. Similar to this finding, a 4-year retrospective longitudinal study in Korea showed that a high baseline TyG index was related to T2DM development regardless of other risk factors such as obesity (Lee et al., 2016). Therefore, by looking at the baseline TyG index value, it might be possible to predict the risk of DM. These findings in general strengthen our observation that IR/DM can exist in HIV patients before initiation of HAART indicating the association of HIV infection with insulin resistance.

The observation of insulin resistance was also noted to be faster in the current study showing that it can occur very early since an increasing rate was observed in four months. This finding is supported by a studies which who reported the prevalence of IR among patients who were more than one year on HAART to not be significantly higher compared to those who were on shorter treatment (Miguel et al., 2015). Therefore, it can be said that it takes shorter time for HIV infected patients to develop insulin resistance.

In addition to this, the TyG index kept increasing from baseline to week 16. This elevation may indicate the potential for the progression of IR to fully developed diabetes in consecutive years. In support of this, a study showed an increased risk of T2DM with increasing TyG index in normal-weight individuals (Ming et al., 2015).

In the present study, difference in the mean plasma glucose between treatment groups was not significantly different. Similarly, the plasma glucose value showed no abnormality to flag hyperglycemia. However, despite the normal glucose profile, the occurrence of IR was profound. In support of this finding, studies have shown that IR exists before blood glucose abnormalities. For example, one study conducted on individuals with normal oral glucose tolerance indicated the occurrence of IR in approximately 25% of them (Reaven et al., 1988).

Similarly a study suggested that hyperinsulinemia and IR occur prior to the occurrence of blood glucose abnormalities (Groop et al., 2000). During the course of the disease, IR was the initiating factor in the majority of patients with T2DM. In these conditions, deterioration of glucose tolerance can only be prevented if beta cells increase insulin secretory response and maintain a state of chronic hyperinsulinemia. When this is not achieved, an increase of blood glucose

inevitably occurs. Therefore, Groop suggested that the process of T2DM to be divided into three phases: hyperinsulinemia stage, prediabetes stage and diabetes stage. Similarly, a study conducted by De Fronzo et al. strengthens the evidence by suggesting that insulin resistance precedes diabetes for several years though it was not explained the year it takes for IR to develop into a profound diabetes (De Fronzo et al., 2009)

In comparison to the HIV treatment group, a mild elevation in the overall average random plasma glucose level was observed in patients co-treated with RIF based anti TB medications and EFV based ART. The reason for the difference might be due to the hyperglycemic effect of the two anti TB medications rifampicin and isoniazid as reflected by previous studies (Asfandyar et al., 2012, Girling et al., 1982 and Takasu et al., 1982).

The present study measured the Random Blood Glucose (RBG) as a diagnostic tool for hyperglycemia. In the TB-HIV co-treatment group, the mean blood glucose was higher than the HIV treatment group. Despite the increase, no association between RBG value and IR was observed. In contrary to this finding, a study conducted on non-fasting participants without diagnosed diabetes has indicated a single RBG $\geq$ 100 mg/dL to be more strongly associated with undiagnosed diabetes than any single risk factor. This finding remained strongly associated with undiagnosed diabetes after adjustment for traditional diabetes risk factors. According to the study, an elevated RBG was more strongly associated with undiagnosed diabetes than the United States Preventative Services Task Force guidelines and was similar to American Diabetes Association guidelines (American Diabetes Association, 2018 and Michael et al., 2015). The reason for the difference with the present study might be related to study participants. The present study included HIV infected patients unlike HIV uninfected patients. In HIV infected patients, additional risk factors may contribute to IR rather than hyperglycemia.

In the present study, no association between liver enzymes and IR was observed. In contrary to this, few studies have shown increased activities of liver enzymes especially aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to be indicators of hepatocellular injury and that increased activity of these markers was associated with insulin resistance (Wannamethee et al., 2005 and Nannipieri et al., 2005). The difference might be related to selection of study subjects. In those studies, non HIV infected study participants were enrolled.

In HIV infected population, other HIV or HIV therapy related risk factors may contribute to IR than liver enzyme abnormalities.

No significant association between IR and lipid profile was noted in HIV only treatment group. However, baseline triglyceride was associated with baseline IR in TB-HIV co-treatment group. In addition to this, the laboratory findings of plasma triglyceride, LDL and HDL have not significantly violated the normal range. In other words, these values are not extremely elevated. In contrary to this finding, features of dyslipidemia included severe hypertriglyceridemia, low levels of high-density lipoprotein (HDL) cholesterol and elevation of low-density lipoprotein (LDL) cholesterol (Calmy et al., 2007). In that study, the dyslipidemia pattern was extremely atherogenic and, coupled with other factors present in HIV patients such as insulin resistance and vascular inflammation, and was believed that it puts these patients at increased risk for premature cardiovascular disease. Therefore, more studies are needed to clearly indicate highly raised lipid profile tests with IR in HIV infected patients.

Dyslipidemia is a common feature among HIV-infected patients, particularly during ART. According to the American Diabetes Association (ADA), all overweight patients whose HDL-cholesterol (HDL) values are <35 mg/dL and whose triglyceride (TG) values are >250 mg/dL should undergo testing for diabetes (American Diabetes Association, 2018). ADA suggests this screening for overweight patients. However, keeping in mind that most HIV patients have a normal BMI, the test might look unpractical for HIV patients. However, few studies have indicated that the high TG and low HDL that are frequently found in HIV infected patients on ART are not always associated with obesity. This is because HIV infected patients often have lower BMIs compared with the general population (Brown et al., 2009).

The mean lipid profile tests have shown an increasing trend from baseline to week 16. This is due to the effect of ART on lipid metabolism. This finding is similar to few studies which reported that in HIV positive patients, the introduction of ART determines an increase in all the lipid profile setting values (De wit et al., 2008 and Brown et al., 2009).

In the present study, baseline triglyceride was a predictor of IR in TB-HIV co-infected patients. This finding was observed in normal triglyceride value. However, this association was inconsistent at weeks 4 and 16. To the knowledge of the researcher, no published articles have

found an association between IR and normal plasma triglyceride value. In contrary to this, studies reported an association between IR and hypertriglyceridemia (Calmy et al., 2007, De wit et al., 2008 and Brown et al., 2009). Therefore, more prospective studies should be conducted to look for the association between normal triglyceride value and IR. In addition to this, the present study showed that the increment in plasma LDL values did not show any form of significant abnormality so does no association between LDL and IR was observed. This finding is similar to that of Rafael *et al.,* which reported the situation where plasma LDL cholesterol levels remain normal in diabetic dyslipidemia (Rafael et al., 2009).

Regarding the HAART regimens involved in the present study, it can be said that elevated TyG index, might not be associated with nucleoside reverse transcriptase inhibitors zidovudine, stavudine, tenofovir or lamivudine because the rate of insulin resistance was not significantly different between patients despite the mean differences. More importantly, the responsible drug for the difference might be the non-nucleoside reverse transcriptase inhibitor efavirenz since insulin resistance existed independent of the nucleoside reverse transcriptase inhibitor presence namely zidovudine, lamivudine, stavudine and tenofovir.

Similar to the present study, a study by Susana *et al* discovered that the incidence of IR among patients taking ART to be higher where, most of the IR was observed in patients taking first-line regimens including efavirenz (Susana et al., 2014). This is also described by Steve *et al* where patients on efavirenz developed IR despite same degree of metabolic abnormalities observed in patients receiving nevirapine based regimens (Steve et al., 2016). In another study the use of nelfinavir in combination with a pair of NRTI was not found to induce insulin resistance more than efavirenz did (Rasmussen et al., 2012). In addition, another study showed that efavirenz containing regimens have a less favorable glucose insulin profile (Shahmanesh et al., 2004).

A study conducted in South Africa also found that EFV was significantly associated with impaired glucose tolerance (Kabati et al., 2010). Participants in that study had all been receiving EFV for at least 6 months at the time of enrolment, suggesting that EFV may accelerate IR.

The role of nucleoside reverse transcriptase inhibitors on IR was not found in the present study. The reason for this is reflected by the short study period. For instance, a study conducted in South Africa studied HIV infected patients who had spent a median of 16 months on first line

ART such as stavudine, zidovudine and nevirapine. This study concluded that long term exposure to ART in these patients was associated with increases in glucose and insulin levels (Zulfa et al., 2015). Therefore, a longer study period is required to look for an association between IR and nucleoside reverse transcriptase inhibitors. This finding was supported by a study conducted in the United States in HIV infected women receiving HAART (Phyllis et al., 2008). According to that study, cumulative exposure to nucleoside reverse transcriptase inhibitors (NRTIs) of >3 years was associated with IR and was higher than the IR without any cumulative NRTI exposure. Similarly, cumulative exposure to the NRTI stavudine of >1 year was associated with significant IR higher than the IR observed without any cumulative stavudine use (Phyllis et al., 2008). The study concluded that longer cumulative exposure to NRTI; in particular, stavudine was associated with greater IR in HIV-infected women. In the present study, no association between stavudine use and IR was observed. The difference for this might be explained by the short duration of study period in the present study. More importantly, in that study no elevated association between cumulative exposure to lamivudine, zidovudine, or tenofovir and IR was substantial (Phyllis et al., 2008). This finding is similar to the current study.

Previous studies have indicated a higher risk of IR in male patients (Ledergerber et al., 2007, De wit et al., 2008, Phyllis et al., 2008 and Kalra et al., 2011). Similar to this, the present study noted a higher TyG index in male study participants of both treatment groups even though no significant association between gender and IR was observed. In contrary to this, a study conducted in china found a higher rate of IR in females than in males (Shao-Jie et al., 2018). Numerous genetic polymorphisms have been associated with pancreatic beta cell function and related to peripheral IR. Some of these polymorphisms were strictly race-dependent, and others have a transethnic association. Therefore, those differences may be due to the effects of race and ethnicity on glucose homeostasis (Bianco et al., 2015). In addition, the relatively small number of male participants in the present study might contribute to this difference.

In the present study a higher mean TyG index was observed in patients aged 40 and above in both treatment groups. Despite this mean difference, there was no statistically significant association between advanced age and IR. However, few studies indicated age above 40 as a risk factor for IR (Ledergerber et al., 2007, De wit et al., 2008, Phyllis et al., 2008 and Kalra et al., 2011). The reason for the difference might be explained by the small number of patients aged 40

and above. In the present study, 40 out of 60 patients aged below 40. This might have negatively affected the association.

The present study found a higher mean TyG index in participants having a baseline BMI of less than 18.5kg/m^2. Similarly, a study conducted in China revealed that a low body weight (BMI < 18.5) to have a high risk for IR (Shao-Jie et al, 2018). Low body weight primarily reflects the losses of adipose tissue and/or lean tissue. Adipose tissue plays a central role in regulating energy metabolic and glucose homeostasis through its secretion of various bioactive proteins (Luo et al., 2016). In contrary to this a study indicated a higher BMI to be associated with IR (Ledergerber et al., 2007, De wit et al., 2008, Phyllis et al., 2008 and Kalra et al., 2011). Further studies are needed to provide a better understanding for the relationship between BMI and IR among IFG participants.

In the present study, a higher mean TyG index was observed in patients with HIV stage 4 infection. No significant association between advanced HIV stage and IR was also observed. Few studies found advanced HIV infection to have an association with higher rate of IR (Fitchenbaum et al., 2005, Kalra et al., 2011 and Gutierrez et al., 2012). In contrary to this finding, few studies witnessed that HIV infection itself irrespective of HIV stage was associated with IR (Dube, 2000, Brown et al., 2005). This finding is similar to the present study.

Few studies have indicated that a lower CD4 cell count below 200cells/mm^3 was a risk factor and predictor of IR in HIV infected patients (Dube, 2000, Fichtenbaum et al., 2005 and Gutierrez et al., 2012). In contrary to this, the present study found no significant association between a lower CD4 and IR. Therefore, more prospective studies which assess the relationship between a lower CD4 cell count and IR should be conducted to clear the issue.

For the present study, only baseline viral load was measured. The baseline viral load was not significantly related to insulin resistance as evidenced by chi square, one-way ANOVA and binary logistic regression analysis. Similarly, findings of the relation between baseline viral load and insulin resistance was not reported for the present study because an increase in viral burden of 0.5 log over a 6-month period, rather than a baseline viral load was assumed to be a risk factor for development of insulin resistance in HIV infected patients (Fichtenbaum et al., 2005, Kalra et al., 2011 and Gutierrez et al., 2012).

6. Limitations of the study

Despite the fact that the present study tried to investigate a very sensitive issue which has a practical importance to developing countries and tried to identify the problem with newer and cost-effective surrogate markers; the findings should be interpreted taking the limitations into consideration.

It could have been excellent to measure plasma insulin value to better predict the risk of insulin resistance but we failed to study plasma insulin value for two reasons. The first is plasma insulin value cannot be measured on a fed state. Secondly, the test is too expensive.

Another limitation to our study was its relatively small sample size, which may not adequately show the association. Furthermore, all of our patients were enrolled in few sites. Therefore, our results may have reduced generalization and may not portray the rate of insulin resistance in HIV-infected patients nationally.

Most of our patients were young (mean age ~35-37years), which may have reduced insulin resistance incidence rate.

The maximal duration of study for HIV only group and TB-HIV group was 4 months only. This might be too short to generalize for the whole duration of treatment.

7. Conclusion

A high incidence of IR was observed in both HIV only treatment and TB-HIV cotreatment groups without significant difference between them. Higher mean TyG index was observed in patients in advanced HIV stage in both treatment groups. Baseline triglyceride was a predictor of IR in TB – HIV coinfected cohorts. Insulin resistance was present in both HIV only and TB-HIV coinfected patients taking ART independent of HIV and non HIV related risk factors.

8. Recommendations

The TyG index test, which is an indicative of insulin resistance, should be implemented in health care facilities because the test is practically possible. More importantly, it helps for early identification of IR before patients develop overt diabetes.

Health professionals should screen all HIV patients on HAART for diabetes. This should be implemented as part of the routine test and is suggested to screen these patients at least when they arrive for an ART refill. This prevents patients from developing sudden diabetes related complications. HIV patients diagnosed with diabetes should be treated using the standard treatment guideline set for the general population.

In addition, future researchers should

- Undergo IR tests on blood samples taken from patients on a fasting state.
- Undergo plasma insulin test for better outcomes if financial capacity is possible
- Conduct a study with larger sample size for adequate generalizations.
- Conduct the study in patients representing all age groups.
- Conduct a prospective comparative study lasting for more than 4 months.

Future prospective studies are needed to elucidate the correlation between ART use and IR and assess the importance of pharmacological and non-pharmacological interventions to prevent patients with insulin resistance from developing diabetes.

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