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Insulin Resistance Associated with Impaired Fasting Glycaemia in Anti-Retroviral Therapy Patients

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Abstract

Background: Insulin resistance was associated with type-2 diabetes mellitus regardless of HIV status. However, an association of insulin resistance in prediabetes with HIV-infected subjects undergoing anti-retroviral therapy (ART) has been reported in very few studies.

Methods: Serum insulin and insulin resistance (HOMA-IR) were measured in ART patients with impaired fasting glycemia (IFG) and those with normal glucose tolerance (NGT) along with other biochemical parameters.

Results: There was increased insulin resistance in the ART IFG group when compared to their ART NGT counterparts. There was a positive correlation of HOMA-IR with serum creatinine, triglycerides, and ALT levels. However, serum HDL showed a negative correlation with HOMA-IR.

Conclusion: Present study showed that there is association of insulin resistance in IFG associated with HIV-infected subjects on ART. Hence, monitoring the insulin resistance in ART patients may help in early prevention of type 2 diabetes mellitus.

Keywords: Human Immunodeficiency Virus, anti-retroviral therapy, impaired fasting glycemia, serum Insulin, HOMA-IR

Introduction

The prevalence of adult HIV cases in India had decreased to 0.21 in 2021 since its epidemiological peak in the year 2000 [1]. In India, the census of people living with HIV is estimated to be approximately 24 lakhs and annual new infection was 62.97 thousand in 2021 [1]. Use of multiple drugs acting on different viral targets, known as highly active antiretroviral drugs (HAART) is being used currently to control and decrease the viral load. Antiretroviral therapy has made HIV into a chronic condition rather than a life-threatening condition but the long-term drug exposure causes adverse side effects. Long-term exposure has been shown to cause lipodystrophy and hyperlipidaemia [2]. A few major toxicities associated with long-term antiretroviral therapy (ART) use are hepatotoxicity, lactic acidosis, hyperlipidaemia, insulin resistance and diabetes mellitus [2].

Prediabetes is an intermediate stage between normoglycemia and diabetes mellitus. Diagnostic criteria for prediabetes are based on three test values: fasting plasma glucose level and/or an

OGTT testing with a 2-hour post 75g glucose ingestion value and/or an HbA1c reading. The commonest change in prediabetes is impaired fasting glucose (IFG) with a fasting glucose value of 101-126 mg/dL [3][4]. Previous studies indicate that individuals undergoing ART are more prone to develop prediabetes and proceed to frank diabetes mellitus in the long run [5]. The prevalence of prediabetes in healthy individuals is estimated to be 10.3% in the largest study conducted across 15 states [6]. However, there was no study estimating its prevalence among individuals undergoing ART in India.

Various factors may contribute to increased insulin resistance in HIV-positive patients undergoing ART which include obesity, genetic influences, physical inactivity, and antiretroviral drugs. A study conducted in the US population showed that approximately 30% of HIV-infected patients have prediabetes; which was lower than that of prediabetes regardless of HIV infection [7]. Studies also reported the higher risk of developing impaired glucose tolerance or lipid disorders is high in HIV patients undergoing ART. HIV-infected adults on ART had a 5-fold greater risk of developing diabetes mellitus when compared to HIV-negative individuals [8]. This may further be responsible for a higher prevalence of impaired fasting glucose and impaired glucose tolerance among them.

The present study aimed to associate insulin resistance in patients undergoing ART and prediabetes and ART patient with normoglycemia and correlate it with biochemical parameters.

METHODOLOGY

Study design:

Subjects who were clinically diagnosed as HIV positive and on ART were recruited for this cross-sectional study. They were recruited from an ART center of a tertiary care center in Western India. The Institutional Ethical Committee had approved the study and written informed consent was obtained from all subjects recruited in the study. Based on the fasting glycemic status, the participants were segregated into two groups as stated in the American Diabetes Association Criteria (2021) [4]. By the inclusion/exclusion criteria, 80 subjects each were recruited in the two groups: ART IFG and ART normal glucose tolerance (NGT), consecutively; within a period from January 2022 to October 2022. The inclusion criteria for this study consisted of participants who were aged between 18 and 55 years, HIV infected for more than 12 months, and who were on first-line ART. Subjects with co-morbidities such as T2DM, hypertension, dyslipidemia, coronary artery disease, malignancy, or chronic renal failure present before starting ART or those who were non-adherent to ART were excluded from this study.

Sample Collection:

Patients were advised to do a 12-hour overnight fasting, followed by which 5mL blood was collected from each subject and segregated into two vacuum evacuated tubes as follows: 3mL of blood was collected in a yellow cap tube containing separating gel for biochemistry analysis and insulin measurements, and 2mL of blood was collected in a grey-colored sodium fluoride tube for glucose estimation.

Biochemical Parameters:

Biochemical analysis was carried out in a clinical lab accredited as per ISO 15189:2012. The biochemical parameters were analyzed using standard methods: Fasting Blood Glucose (FBG) using the hexokinase method, Blood Urea Nitrogen (BUN) using the Urease GLDH method, serum creatinine using the Jaffes Kinetic method, serum triglycerides (TG) using glycerol kinase-glycerol 3 phosphate oxidase-peroxidase method, serum total cholesterol using cholesterol esterase-cholesterol oxidase-hydrogen peroxidase method), high-density lipoprotein-cholesterol (HDL-c) using the direct method, total bilirubin (TB) using Diazo Sulfanilic Method, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using the UV kinetic (IFCC) method, total protein (TP) using the Biuret method, serum albumin using BCP Dye Binding method. Siemens Dimension EXL-200 auto

analyzer was used for measurements of these biochemical parameters. Low-density lipoprotein-cholesterol (LDL-c) was calculated using the Friedewald formula by the auto analyzer.

Serum Insulin estimation by Enzyme Linked Immuno Sorbent Assay (ELISA):

Quantitative Sandwich ELISA was used for the estimation of serum insulin levels and quantified using a Calbiotech Insulin ELISA kit (Cat No-PI099D). Standards and samples were pipetted into the wells of a microplate which were precoated with monoclonal antibodies specific to insulin and ELISA was performed as per manufacturer's instruction. The color developed was measured at 450nm and serum Insulin values were expressed in µIU/mL. Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) was used to determine Insulin Resistance (IR) using the formula: fasting insulin (µIU/mL) x fasting glucose (mg/dL)/405.

Statistical analysis:

GraphPad Prism version 9.4.1 was used for the statistical analysis of data. The Shapiro-Wilk test was used to detect the normality of continuous variables. Numerical data were expressed as median with interquartile range (IQR). For variables that were not normally distributed, non-parametric tests were used. Mann-Whitney test was used for the comparison of two groups namely: ART NGT and ART IFG. The Spearman correlation test was used for the correlation analysis. A p-value of <0.05 was considered significant.

RESULTS

A total of 160 patients were included in this study. Table 1 shows the results of the biochemical analysis of the ART NGT group and ART IFG. Age, fasting glucose and HOMA-IR were the only parameters showing significant differences between the NGT and IFG groups. The fasting glucose level was significantly higher (p=<0.0001) in the ART IFG group (median=106.5mg/dL, IQR=8.25) as compared to the ART NGT group (median=93.50, IQR=7.0). Correspondingly, the HOMA-IR of the ART IFG group (median=5.1, IQR=3.63) was significantly higher (p=0.0011) than that of the ART NGT group (median=4.5, IQR=2.26). All other parameters showed no statistical significance.

Table 1: Biochemical parameters of patients with NGT and IFG

Parameters	NGT	IFG	P value
Age	36.0 (14.25)	36.0 (14.00)	0.0249
Fasting	93.50 (7.00)	106.5 (8.25)	***<0.0001***
BUN	9.00 (5.00)	8.00 (4.00)	0.4002
Creatinine	0.70 (0.30)	0.70 (0.30)	0.8778
TG	99.00 (59.75)	90.00 (65.50)	0.6066
Cholesterol	165.5 (60.00)	183.5 (40.00)	0.0964
HDL	47.00 (18.25)	49.00 (19.25)	0.6710
LDL	99.50 (55.85)	109.5 (28.75)	0.2700
TB	0.40 (0.30)	0.40 (0.20)	0.3827
ALT	31.50 (12.25)	29.00 (12.00)	0.1837
AST	26.00 (8.25)	26.00 (9.25)	0.5277
TP	8.0 (0.60)	8.1 (0.75)	0.4545
ALB	3.9 (0.40)	4.0 (0.40)	0.0594
Insulin	19.6 (9.73)	19.6 (13.25)	0.2826
HOMA IR	4.5 (2.26)	5.1 (3.63)	***0.0011***

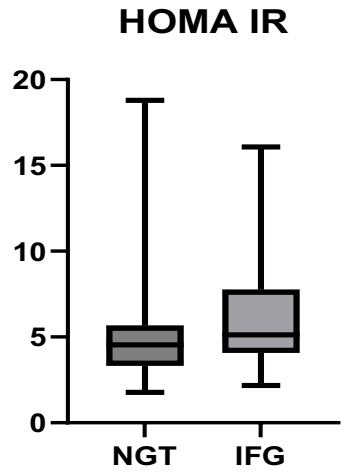

Figure 1: HOMA IR of ART-IFG and ART-NGT patients

Correlation between different parameters:
Table 2 shows the result of the correlation analysis between different biochemical parameters and HOMA-IR of the ART IFG group. Out of all the biochemical parameters serum creatinine level (p=0.0265), serum triglycerides (p=0.0072), HDL cholesterol (p=0.0345), and Alanine aminotransferase (p=0.0146) are the only parameters showing significant correlation with HOMA-IR. Serum Creatinine level (r=0.248, 95% CI= 0.023 to 0.449), Serum triglycerides (TG) (r=0.299, 95% CI= 0.078 to 0.491) and ALT levels (r=0.272, 95% CI= 0.049 to 0.469) showed a positive correlation with HOMA-IR. However, serum HDL cholesterol (r=-0.237, 95% CI= -0.439 to -0.011) showed a negative correlation with HOMA-IR.

Table 2: Correlation analysis of different biochemical parameters with HOMA IR.

Parameters	**HOMA-IR**	**p-value**
	R value (95% CI)	
Age	0.203 (-0.024 to 0.410)	0.0711
Fasting	-0.081 (-0.301 to 0.148)	0.4777
BUN	-0.034 (-0.258 to 0.194)	0.7648
Creatinine*	0.248 (0.023 to 0.449)	***0.0265***
TG*	0.299 (0.078 to 0.491)	***0.0072***
Cholesterol	-0.110 (-0.328 to 0.119)	0.3308
HDL*	-0.237 (-0.439 to -0.011)	***0.0345***
LDL	-0.099 (-0.318 to 0.130)	0.3840
TB	0.035 (-0.192 to 0.259)	0.7563
ALT*	0.272 (0.049 to 0.469)	***0.0146***
AST	0.122 (-0.107 to 0.339)	0.2805
TP	0.054 (-0.174 to 0.277)	0.6313
ALB	0.05306 (-0.175 to 0.275)	0.6402

Discussion

ART drugs such as protease inhibitors or reverse transcriptase inhibitors have been shown to induce insulin resistance, and dyslipidemia, and leads to type 2 diabetes mellitus and cardiovascular risk [2][9]. Another mechanism of insulin resistance in HIV patients includes the increased levels of circulating inflammatory cytokines mediated by the increased levels of LPS which in turn activate TLR4, IL-6, and TNF-alpha receptors, and inflammasome activation. This in turn leads to a downregulation in insulin signalling and causes insulin resistance in these patients [10]. The present study examined the association of insulin resistance among prediabetic individuals undergoing ART as compared to normoglycemic individuals on ART.

The results from the present study are consistent with other previous studies suggesting an increased insulin resistance in prediabetic HIV-positive patients on ART. This was also correlated to certain biochemical parameters whether due to pre-existing comorbidities or as a side effect to anti-retroviral therapy [10]. This data is of clinical significance as tracking the biochemical parameters of HIV-positive patients and closely monitoring the drugs used in combinational antiretroviral therapy may decrease the progress of prediabetes from developing into insulin resistance, however further research is needed to conform the same. To our knowledge, this study is the first of its kind in India to study the association of insulin resistance in prediabetic HIV-positive patients on ART.

The results also showed a significant correlation between biochemical parameters such as serum creatinine, serum triglycerides, serum HDL levels, and serum ALT with the HOMA-IR of impaired fasting glucose individuals with HIV on ART although HDL showed a negative correlation to HOMA-IR. This is consistent with other studies showing elevated total cholesterol, and increased homeostasis model assessment of insulin resistance, from a period of as much as 90 days post-beginning of combination antiretroviral therapy (cART) [11] [12]. Furthermore, the prevalence of high-density lipoprotein is shown to decrease with increasing HOMA-IR which remains consistent with this study [13].

Strengths of this study include a large population size of both ART NGT and IFG HIV-positive groups when compared to previous studies making the power of the study high. We also have used the ELISA technique and HOMA-IR to represent IR. However, there were some limitations in this study, including not performing the OGTT and HbA1c test to confirm prediabetes.

Conclusion

HIV-affected individuals undergoing ART are more susceptible to develop insulin resistance whether as a side effect of ART drugs or due to comorbidities associated with HIV. Hence it is recommended to measure the fasting blood glucose as well as insulin resistance levels in ART patients regularly to monitor and give them better treatment options as early as possible.

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