# Effect of Protease Inhibitors on Serum Glucose Level of HIV Patients Attending Faith Alive Foundation, Jos, Plateau State

Duru BN<sup>1\*</sup>., Yakunat OE<sup>2</sup>., Dalyop KA<sup>2</sup>., Duile PT<sup>2</sup>., Peter L<sup>2</sup>., Okafor PA<sup>3</sup>., Plagnan AG<sup>3</sup>., Tyem DA<sup>3</sup>., Jik WA<sup>3</sup>., Kinjir HJ<sup>3</sup>., Abilu CA<sup>3</sup>., Mohammed SJ<sup>3</sup>., Mukaila FB<sup>3</sup>., Lokason SD<sup>3</sup>., Mohammed M<sup>3</sup>., Timothy V<sup>2</sup>.

[162] (Dept of Chem. Path. FCVMLT/Side Lab. FCAHPT / Viro. / Histo. FCVMLT/Div. of Quality Control NVRI)

[3] (Div. of Cent. Diagnostics, NVRI/Chem. Path. ABU, Zaria/University of Jos/ Bact. Vacc. Prod. NVRI)

[3] (Div. Parasitology/Bact. Vacc. Prod. NVRI/Dept Chemical Path CHT, Kano/Dept. Hema. / BGS, FCVMLT/)

Corresponding Author: Duru Boniface Nnamdi, email: <a href="mailto:aefule4u@yahoo.com">aefule4u@yahoo.com</a> Phone: 08035873744. Department of Chemical Pathology, F C V M L T, PMB 02, Vom. Postcode = 930010

**Abstract:** The serum samples of 100 HIV positive patients on medication (protease inhibitors) and 100 HIV positive patients not on medication (control) attending Faith Alive Foundation, Jos, were investigated for their serum glucose level using Dialab liquid reagent kits. The mean results of the serum glucose level were higher in female test (5.4302) and male test (4.9702) than in control female (4.8403) and control male (4.9435) respectively. The mean comparison was made using one way ANOVA and the result showed that the serum glucose levels of female and male tests against female and male control was not clinically significant (p > 0.05). However Comparison by age group made using the Post Hoc test shows that age group of 41 to 60years and above, shows a clinical significant difference (P < 0.05). This led to the conclusion that protease inhibitors have associated side effects of which glucose is among them and should therefore be monitored in the cause of treatment to prevent hyperglycemia that can lead to diabetes as well as other complications.

**Key words:** HIV, Glucose, Protease, Protease Inhibitors, Diabetes, Hyperglycemia.

#### I. Introduction

Blood sugar is the amount of glucose in the blood. Glucose, transported via the bloodstream, is the primary source of energy for the body's cells. Blood sugar concentration, or glucose level, is tightly regulated in the human body. Normally, the blood glucose level is maintained between about 4 and 6 mmol/L. Normal blood glucose level (homoeostasis) is about 90mg/100ml or 5mM. The total measurement of glucose in the circulating blood is therefore about 3.3 to 7g (assuming an ordinary adult blood volume of 5 liters)[1]. Glucose levels rise after meals and are usually lowest in the morning, before the first meal of the day. Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycemia) or low (hypoglycemia) blood sugar. Only glucose levels are regulated via insulin and glucagon[1]

Two types of HIV exist: HIV-1 and HIV-2. HIV-1 is more virulent, is more easily transmitted and is the cause of the vast majority of HIV infections globally [2]. The pandemic strain of HIV-1 is closely related to a virus found in the chimpanzees of the subspecies Pan troglodytes troglodytes, which lives in the forests of the Central African nations of Cameroon, Equatorial Guinea, Gabon, Republic of Congo (or Congo-Brazzaville), and Central African Republic. HIV-2 is less transmittable and is largely confined to West Africa, along with its closest relative, a virus of the sooty mangabey (Cercocebus atys atys), an Old World monkey inhabiting southern Senegal, Guinea-Bissau, Guinea, Sierra Leone, Liberia, and western Ivory Coast[2][3]. Most HIV patients have been found to develop a diabetic condition primarily out of the medications prescribed in the treatment of the HIV complication. Since this onset of abnormal blood glucose is considered Type II diabetes, the patient can usually manage the irregularity with diet and exercise, occasionally requiring the use of insulin injections [1]. The economic costs of diabetes globally was estimated in 2013 at \$548 billion [16] and in the United States in 2012 \$245 billion[4].

Protease inhibitors (PIs) are a class of medications used to treat or prevent infection by viruses, including and Hepatitis C. PIs prevent viral replication by inhibiting the activity of HIV-1 protease, an enzyme used by the viruses to cleave nascent proteins for final assembly of new virons figure 1. Protease inhibitors have been developed or are presently undergoing testing for treating various virus; HIV/AIDS: antiretroviral protease inhibitors (saquinavir, ritonavir, indinavir, nelfinavir etc.) [5]. All protease inhibitors work in the same manner. They block the activity of the protease enzyme within human cells or new virus particles. This enzyme is what allows a virus to undergo final maturation figure4 for producing new viruses that can go out and infect other cells. When the PIs bind to the enzyme, the new viruses still leave the cell, but they are unable to infect other

cells [6].. HIV is a virus that goes through many steps during it's' life cycle figure 2. Once HIV infects a human cell, the virus uses proteins and chemicals inside that cell to make more copies of it. Protease figure 1 is a chemical, known as an enzyme that HIV needs in order to make new viruses [6].

If untreated, eventually most HIV-infected individual develop AIDS (Acquired Immunodeficiency Syndrome) and die; however, about one in ten remains healthy for many years with no noticeable symptoms . [7]. Treatment with anti-retroviral, where available, increases the life expectancy of people infected with HIV. Protease inhibitors (PIs) block the protease enzyme. When protease is blocked, HIV makes copies of itself that can't infect new cells [8]. Studies have shown that protease inhibitors can reduce the amount of virus in the blood and increase CD4 cell counts. In some cases these drugs have improved CD4 cell counts, even when they were very low or zero [9] [10].

# 1.1 Justification

Most HIV patients commonly experience greater degrees of blood glucose fluctuations and are at a greater risk for the secondary complications associated with abnormal blood glucose, including infection due to the medications and the therapies used to treat them, but little or no information are available in this part of the country on these complications.

# 1.2 Aims And Objectives

- 1. To estimate the level of serum glucose in HIV patients on medication (Protease Inhibitor)
- 2. To estimate the level of serum glucose in HIV patients without medication (Protease Inhibitor).
- 3. To compare the levels of glucose in patients on medication with patients not on medication.

#### II. Material And Method

# 2.1 Sample Collection

A total of 200 samples were collected from Faith Alive Foundation, Jos Plateau State. The samples were collected from both sexes within age 20-60 out of this number, 100 was collected from HIV patient on medication (Protease Inhibitors) and 100 from HIV patient not on medications. 2ml of venous blood was collected by venipuncture after sterilizing the Cubital Fossa with methylated spirit and avoiding venous stasis. Each sample was transferred into an appropriately labeled clean dry sample bottles. This was allowed to stand for 30minutes for it to clot and retract. It was then centrifuge at 3000 revolution per minute (rpm) for 5minutes. The serum was estimated immediately and the absorbance reading was taken at 500nm.

#### 2.2 Inclusion criteria

HIV patients only

HIV patients on protease inhibitor only

HIV patients who has not developed AIDS

HIV patients on protease inhibitor from two weeks and above.

HIV patients with CD4+ count of two hundred and fifty and above

# 2.3 Exclusion criteria

Non HIV positive patients

HIV patients on drugs other than protease inhibitor

HIV patients who has developed AIDS

HIV patients less than two weeks on protease inhibitor

HIV patients with CD4+ count of less than two hundred and fifty

# 2.2 Method: Estimation Of Serum Glucose Using Dialab Liquid Reagent.

# 2.2.1 Procedure

 $1000\mu l$  of the reagent was pipetted into dried cleaned test tubes labeled Sample, Blank and Standard each.  $100\mu l$  of the sample and standard was added to the reagents and distilled water was used for the blank to make up the volume. It was mixed and incubated for 10minutes at 37°C and absorbance reading was taken at 500nm.

# Calculation:

Glucose concentration = Abs. of Test × Conc. of Std.

Abs. of Std.

III. Result
Table 1 Comparison Of Serum Glucose Level Between Sex For Both Tests And Controls.

	N Mean S/Deviation	p-value	Remark
Male (test)	47 4.9702 1.4104	0.22	not significant
Female (test)	53 5.4302 1.5289		
Male	23 4.9435 1.5678		
(Control)			
Female	77 4.8403 1.7959		
(Control)			
TOTAL	200 5.0390 1.6225		

Table 2 Comparison Of Serum Glucose Level Between Ages Of The Test.

	N	Mean	S/Deviation	p-value	Remark
21-30	31	5.2355	1.4084	0.57	not significant
31-40	36	5.0361	1.5143		
41-50	17	5.6471	1.6838		
51-60	16	5.1125	1.3827		
TOTAL	10	0 5.2140	1.4850		

Table 3 Comparison Of Serum Glucose Level Between Age Group Of The Test.

	N	Mean	S/Deviation	p-value	Remark	
21-30	55	4.8218	1.7104	0.03	significant	
31-40	26	4.5308	1.6240			
41-50	15	5.9200	1.7514			
51-60	4	3.6500	1.5199			
TOTAL	100	0 4.864	10 1.7390			

Table 4

Multiple Comparisons

Dependent Variable: GLUCOSE

LSD

		Mean Difference			95% Confidence Interval	
(I) SEX	(J) SEX	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
male (test)	female(test)	4600	.32391	.157	-1.0988	.1788
	male(control)	.0267	.41139	.948	7846	.8380
	female(control)	.1300	.29925	.665	4602	.7201
female(test)	male (test)	.4600	.32391	.157	1788	1.0988
	male(control)	.4867	.40366	.229	3094	1.2828
	female(control)	.5899*	.28854	.042	.0209	1.1590
male(control)	male (test)	0267	.41139	.948	8380	.7846
	female(test)	4867	.40366	.229	-1.2828	.3094
	female(control)	.1032	.38415	.788	6544	.8608
female(control)	male (test)	1300	.29925	.665	7201	.4602
	female(test)	5899*	.28854	.042	-1.1590	0209
	male(control)	1032	.38415	.788	8608	.6544

 $<sup>\</sup>ensuremath{^{\star}}\xspace$  The mean difference is significant at the .05 level.

### **Post Hoc Test**

Table 5

Multiple Comparisons

Dependent Variable: GLUCOSE

LSD

		Mean Difference			95% Confidence Interval	
(I) Age group	(J) Age group	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
20-30	31-40	.2910	.40192	.471	5068	1.0889
	41-50	-1.0982*	.49191	.028	-2.0746	1217
	51-above	1.1718	.87455	.183	5641	2.9078
31-40	20-30	2910	.40192	.471	-1.0889	.5068
	41-50	-1.3892*	.54755	.013	-2.4761	3023
	51-above	.8808	.90701	.334	9196	2.6812
41-50	20-30	1.0982*	.49191	.028	.1217	2.0746
	31-40	1.3892*	.54755	.013	.3023	2.4761
	51-above	2.2700*	.95032	.019	.3836	4.1564
51-above	20-30	-1.1718	.87455	.183	-2.9078	.5641
	31-40	8808	.90701	.334	-2.6812	.9196
	41-50	-2.2700*	.95032	.019	-4.1564	3836

<sup>\*.</sup> The mean difference is significant at the .05 lev el.

#### IV. Discussion

The result obtained from the work done shows that the glucose level of the HIV patients that are positive and on medication was higher than those not on medication. The average glucose level of the male test and control were 4.97 and 4.94 respectively and the female test and control were 5.43 and 4.84 respectively. The glucose level in test male and female were therefore higher than control. This is in agreement with the study carried out by [11][12][13][14] [15] [16] which states that there was relatively rapid development of insulin resistance after the initiation of protease inhibitor therapy and when evaluated, fasting insulin and glucose levels as well as insulin resistance increased significantly. Also in another work done by [17][18]. first-generation protease inhibitors, including the drug ritonavir, block GLUT4, a protein that transports glucose from the blood into the cells where it is needed. This raises blood sugar levels — a hallmark of diabetes.

In table 1, it was observed that comparison of mean serum glucose between the male and female test against male and female controls was not significant. This may probably be due to the fact that the side effect of the drug on the patients have not begun to show and genetic factors have also been implicated in the way an individual responds to side effect of any drug visa vis: absorption, distribution and elimination of the drug[19]. This was also observed in table 2 in which comparison based on age group was seen to be generally not significant p>0.05. But comparison within the age group of the tests showed that age group 41-50 with mean (5.647) was higher than the other age group and significant p<0.05.

There was a significant difference between pair wise comparison as well as post hoc tests of female test and female control while male test and male control had no such significant difference p>0.05 tables 3, 4, and 5 which may be due to the fact that men demonstrated an increased rate of insulin clearance and increased peripheral tissue sensitivity to insulin when compared to non HIV- infected controls[20] In addition diabetes mellitus is associated with such long-term complications as retinopathy figure 3, nephropathy, and neuropathy. It currently accounts for more cases of loss of vision, renal failure, and amputation figure 4 than any other disease [21]. However, even more importantly, persons with diabetes mellitus have two to five times the risk of cardiovascular disease than persons without the disease and the majority of patients with diabetes mellitus die of cardiovascular disease. Furthermore the Food and Drug Administration warned doctors to closely monitor patients on protease inhibitors because of reports of hyperglycemia and diabetes mellitus associated with protease inhibitors. After this warning, physicians continued to report cases of hyperglycemia and diabetes mellitus in association with protease inhibitors. Two retrospective studies suggested that the incidence of newonset diabetes mellitus after initiation of protease inhibitor therapy is between 6% and 7% [13][22] found that HIV-infected persons taking the protease inhibitor indinavir for only a few weeks developed fasting hyperglycemia and decreased insulin sensitivity, typical predictors of future diabetes mellitus [23]

# Conclusion

From the results obtained, the glucose level of the HIV patients on medication (protease inhibitor) was higher than of the HIV patients not on medication and this could result to metabolic disorders such as diabetes mellitus, ketoacidosis, atherosclerosis and other preventable diseases.

#### VI. Recommendation

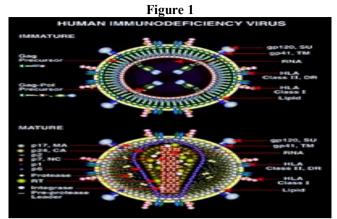
We therefore recommend that glucose levels of HIV patients on protease inhibitors should be closely monitored.

# Acknowledgement

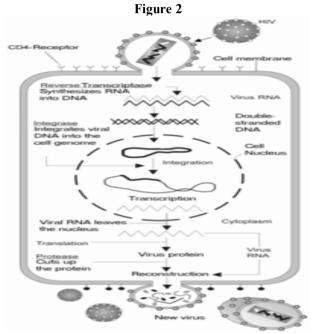
We owe our profound gratitude to Dr Chris Isichie for his acceptance in allowing us access to the patients as well as giving us every other assistance. We are also grateful to members of staff of the foundation for their overwhelming co-operation in carrying out this work.

### Reference

- [1].
- John Bernard Henry, M.D.(2001). Clinical diagnosis and management of by laboratory methods. Reeves JD, Doms RW (2002). "Human immunodeficiency virus type 2". The Journal of general virology **83** (Pt 6): 1253–65. [2]. doi:10.1099/vir.0.18253-0 (inactive 2014-02-04). PMID 12029140.
- Santiago ML, Range F, Keele BF, Li Y, Bailes E, Bibollet-Ruche F, Fruteau C, Noë R, Peeters M, Brookfield JF, Shaw GM, Sharp PM, Hahn BH (2005). "Simian Immunodeficiency Virus Infection in Free-Ranging Sooty Mangabeys (Cercocebus atys atys) from [3]. the Tai Forest, Cote d'Ivoire: Implications for the Origin of Epidemic Human Immunodeficiency Virus Type 2". Journal of Virology 79 (19): 12515–27. doi:10.1128/JVI.79.19.12515-12527.2005. PMC 1211554. PMID 16160179
- [4]. American Diabetes, Association (Apr 2013). "Economic costs of diabetes in the U.S. in 2012." Diabetes care 36 (4): 1033-46. PMID 23468086.16
- [5]. FDA (Food and Drug Administration), (2006). Grants Fast Track "Hostile takeovers: viral appropriation of the NF-kappaB pathway". Designation To Oral HCV Protease Inhibitor SCH 503034
- James, D.S. (2005). Test Positive Aware Network. The Protease Inhibitor Drug. Curr. Opin. Infect. Dis 3 (8): 314-326
- Buchbinder, S.P., Katz, M.H., Hessol, N.A., O'Malley, P.M., Holmberg, S.D., (1994). "Long-term HIV-1 infection without immunologic progression." AIDS 8 (8): 1123-1128.
- Hézode C (2012) Boceprevir and telaprevir for the treatment of chronic hepatitis C: safety management in clinical practice. Liver [8]. International 32: 32-38.
- Anonymous. (2005). Joint United Nations Programme on HIV/AIDS. AIDS epidemic update, Retrieved on 2006-02-28.
- [10]. Anonymous. (2006). "Protease Inhibitor; A Simple Fact Sheet from the AIDS Treatment Data Network.
- Lumpkin M.M (1997). Reports of diabetes and hyperglyceamia in patients receiving protease inhibitor for the treatment of HiV. Ī11Ī.
- Behrens G, Dejam A., (1999): Impaired glucose tolerance, Beta-cell function and lipid metabolism in HIV patients under treatment [12]. with protease inhibitors. AIDS 13: F63-70.
- [13]. Dever LL, Oruwari P.A, FigueroaW E,(2000): "Hyperglyceamia associated with protease inhibitors in an Urban HIV- Infected monitoring Patient Population". Annals of Pharmacotherapy. 34:580-4.
- [14]. Cecilia Yoon, Roy M Gulick, Donald R Hoover, Marshall J Glesby (2005). Case control study of Diabetes Mellitus in HIV infected patients.AIDS 37 (4): 1466-1469
- [15]. Fantry, LE (2003). "Protease inhibitor-associated diabetes mellitus: A potential cause of morbidity and mortality". Journal of acquired immune deficiency syndromes (1999) 32 (3): 243-4. PMID 12626882
- Lilian F Lien, Mark N Feinglos (2005). Protease Inhibitor induced diabetic complications. Drug safety 28(3): 209-226.
- Vyas A, Koster J, Tzekov A, Hruz, P. Effects of the HIV Protease Inhibitor Ritonavir on GLUT4 Knock-out Mice. Journal of [17]. Biological Chemistry. Nov. 19, 2010.
- [18]. Zheng, Y. H., Lovsin, N. and Peterlin, B. M. (2005). "Newly identified host factors modulate HIV replication". Immunol. Lett. 97
- ]http://www.forcon.ca/learning/hitting.html, 2004
- El-sadr (2005). Increased insulin sensitivity in HIV/AIDS patient on antiretroviral therapy. [20].
- Nathan DM. Initial management of glycemia in type-2 diabetes mellitus. N Engl J Med 2002; 1342-9. [21].
- [22]. Carr A, Samaras K, Thorisdottir A, et al. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and DM: a cohort study. Lancet 1999; 353:2093-9.
- Dube MP, Edmondson-Melancon H, Qian D, et al. Prospective evaluation of the effect of initiating indinavir-based therapy on [23]. insulin sensitivity and B-cell function in HIV-infected patients. J Acquir Immune Defic Syndr 2001; 27:130-4.



The immature and mature forms of HIV



The HIV replication cycle

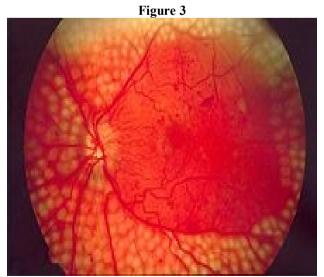


Image of <u>fundus</u> showing scatter <u>laser surgery</u> for <u>diabetic retinopathy</u>



Foot ulcers are a common complication of diabetes and can lead to amputation. This ulcer is further complicated by both wet and dry gangrene.