

The Incidence of Diabetes Mellitus among Human Immunodeficiency Virus (HIV) Positive Patients on Therapy in Nnewi.

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Abstract: Diabetes Mellitus (DM) is a common disorder affecting individuals of all ages. Similar to general population, diabetes mellitus (DM) can also be seen in HIV infected subjects. In this study, 150 confirmed HIV positive subjects, 50 of whom were on the first line drug of Lamivudine, Nevirapine, Combivir, Combipack and efavirenze were recruited. The second group of 50 subjects was on second line drugs which consisted of Truvada and Aluvia. Truvada is from first line drug and comprises tenofovir and emtricitabine while aluvia is protease inhibitor and comprises lopinavir and ritonavir. The third group of 50 subjects was not on antiretroviral therapy and was used as control. All the patients were screened for diabetes mellitus (DM) before the commencement of antiretroviral therapy and found to be negative. Assay was performed three months after the commencement of therapy. There is a prevalence of 6% diabetes mellitus in those on second line drugs which comprised mostly protease inhibitors compared to 0% of those on the first line drug and control samples using a cut-point of 11.0 mmol/L of random blood sugar as recommended by World Health Organization (WHO). It is possible that the rise in blood glucose level may be a result of Aluvia, a protease inhibitor rather than Truvada a first line drug. There is also a prevalence of 10% impaired glucose intolerance based on WHO cut-point of 8.0mmol/l. Most of the patients recruited for this work had CD4 less than 350 cells/ μ l, which is the WHO cut point before drug can be administered to a patient. Therefore primary care for HIV – infected individuals with reference to DM and its complication is important.

Keywords: Diabetes mellitus, HIV, Anti-retroviral therapy (ART).

I. Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which glucose is underutilized. It is usually due to a combination of hereditary and environmental causes resulting in abnormally high blood sugar levels (hyperglycemia)¹. As the disease progresses, individuals are at increased risk for the development of specific complications including retinopathy (which may lead to blindness), renal failure, neuropathy (nerve damage) and arteriosclerosis. The last condition may result in stroke, gangrene or coronary artery disease². Blood glucose levels are controlled by a complex interaction of multiple chemicals and hormones in the body including the hormone insulin made in the beta cells of the pancreas.

Human immunodeficiency virus (HIV) is a virus associated with disease of immune suppression with long incubation period after infection before manifestation of apparent illness. Infection with HIV results in relentless destruction of the immune system leading to onset of acquired immunodeficiency syndrome (AIDS)^{3,4}. Worldwide, there were about 33 million people living with HIV as at 2007, with 2.7 million newly infected with the virus and 2 million HIV related deaths; 67 percent of this number were in sub-Saharan Africa and 75 percent of deaths from the disease were in the region too^{5,6}. A prevalence of 4.4 percent has been reported within the ages of 15-49 in Nigeria with a total estimate of 2.9 million people infected with the disease^{5,6}. Antiretroviral therapies (ART) are drugs that diminish HIV replication. ART is based on a cocktail of at least three antiretroviral agents (called highly active antiretroviral therapy (HAART) that reduce the viral load, reduce opportunistic infections with almost complete suppression of HIV replication⁷. HAART has markedly improved the prognosis of people with HIV infections for few years⁸. However, there are several clinical and metabolic abnormalities associated with HAART⁹.

In view of this information, the present study was designed to investigate the prevalence of diabetes mellitus in HIV infected patients on ART.

II. Methods

SUBJECTS: The subjects for this study comprised of 150 HIV patients grouped into (i) 50 HIV subjects who were on first line drugs which consisted of a combination of Nucleoside reverse transcriptase

inhibitors (NRTIS); non nucleoside reverse transcriptase inhibitors (NNRTIS) and nucleotide reverse transcriptase inhibitors (NtRTIS); these were called first line patients. (ii) 50 HIV subjects who were on the second line drugs which is the protease inhibitors but all the patients in this group were taking a combination of the first line drug and protease inhibitors mostly of Truvada and Aluvia. Truvada is a combination of Tenofovir and Emtricitabine while Aluvia is a combination of Lopinavir and Ritonavir these were called second line patients while the remaining 50 patients used as control are those not on therapy. These were called no treatment group.

The criteria for the selection of a patient for this study are those that are not diabetic, or have no history of diabetes in their family and have no risk factors of diabetes.

THE BLOOD SAMPLING/ COLLECTION:

5ml of blood sample was collected from each of the patients. 2ml of the blood were dispensed in a fluoride oxalate container and the remaining 3ml of blood were dispensed into a plain bottle for HIV-1 and II screening. All the patients commenced ART within two weeks after testing positive for HIV. The drug doses were according to the natural algorithm. Glucose determination was done after three months of therapy in each of the study group. The patients for analysis were screened for HIV 1 and 2 using Determine, Stat-pak and Uni-gold test kit.

The Principle Of DETERMINE:

Determine HIV - 1 AND 2 is an immuno chromatographic test for the qualitative detection of antibodies to HIV – 1 and 2. Sample is added to the sample pad followed by the addition of running buffer. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient's window site. If antibodies to HIV-1 and or HIV-2 are present in the samples, the antibodies bind to the antigen selenium colloid at the patient window forming a red line at the patient's window site. There should always be a red line at the control window¹⁰.

The Principle of STAT-PAK:

The Chembio HIV-1 and 2 stat-pak assay employs a unique combination of a specific antibody binding protein which is conjugated colloidal gold dye. HIV-1 and 2 antigens are bound to the membrane solid phase. The sample is delivered into the sample well followed by the addition of running buffer. The buffer facilitates the lateral flow of the release products and promotes the binding of antibodies to the antigen; if present the antibodies bind to the gold conjugative antibody binding protein. In a reactive sample, the dye conjugate-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilize in the test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the control area containing immunoglobulin (G) antigens¹¹.

The Principle of UNI-GOLD:

Recombinant proteins representing immunodominant regions of the envelope proteins of HIV-1 and 2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region.

During testing two drops of serum, plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class, specific to the recombinant HIV-1 or HIV-2 proteins, will react with the colloidal gold linked antigens. The antibody colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device.

A positive reaction is visualized by a pink/red band in the test region of the device.

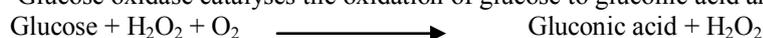
A negative reaction occurs in the absence in the absence of human immunoglobulin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device.

Excess conjugate forms a second pink/red band indicates proper performance of the reagents in the kit⁶.

Glucose concentration in the blood sample was analyzed using glucose oxidase method.

THE GLUCOSE OXIDASE METHOD

Glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide (H₂O₂)



The additional of the enzyme peroxides and a chromogenic oxygen acceptor such as o- toluidine or 4 – amino phenazone results in the formation of a colored compound that is measured at 550nm^{2,12}.

Glucose oxidase is highly specific for b – D glucose. Because 36% and 64% of glucose in solution are in the a and b forms respectively, complete reaction requires mutarotation of a and b forms.

Some commercial preparations of glucose oxidase contain an enzyme glucomutarotase that accelerates this reaction. Otherwise extended incubation time allows spontaneous conversion^{2,12}.

STATISTICAL ANALYSIS: The statistical analysis was performed using students t- test and a P value less than 0.05 (P < 0.05) was obtained indicating a significant difference between those on therapy and those not on therapy.

III. Results

The results showed a zero percent (0%) prevalence of diabetes mellitus on those patients on first line drugs. They also indicated (6%) prevalence of diabetes mellitus and 10% prevalence of impaired glucose tolerance in those on second line drugs.

IV. Discussion

Diabetes mellitus (DM) is a disorder of carbohydrate metabolism. It develops due to diminished production of insulin or resistance to its effect⁸.

The use of highly active antiretroviral therapy (HAART) has remarkably improved long-term survival in HIV infected subjects⁸. Two categories of patients were used in this study. The first category were those on the first line drugs i.e. Nucleoside reverse transcriptase inhibitors (NRTIS), Non-nucleoside reverse transcriptase inhibitors (NNRTIS) and Nucleotide i.e. protease inhibitors (PIs). While the second category are those on first line and second line drugs composed of protease inhibitors.

The study reveals 6% prevalence of Diabetes mellitus (DM) in the ART patients on the second line drug. The 6% prevalence rate was established using a cut- point of 11.0mmol/L according to the WHO recommendation of random blood sugar⁶. About 10% of the patients on the second line drug developed impaired glucose tolerance which suggests a risk of developing diabetes mellitus in future. Yoon *et al*¹³ reported 7% prevalence. Carr *et al*¹⁴ reported that impaired glucose intolerance occurred in 16% of protease-inhibitor recipients and diabetes mellitus in 7% of the 113 patients studied. They concluded that impaired glucose tolerance was common with these drugs. In an earlier report where diabetes developed following treatment protease inhibitors, the authors suggested that a familial disposition may have played a role¹⁵.

The knowledge of the mechanisms responsible for deterioration in glucose tolerance during protease inhibitor containing regimens administration is still not well understood. It is unclear whether protease inhibitors adversely affect pancreatic B – cell function and effect of interaction on glucose metabolism. Although protease inhibitors are end peptidase inhibitors and endopeptidase are involved in the conversion of proinsulin to insulin. The plasma proinsulin level may increase with the administration of protease inhibitors. It follows therefore that these drugs may directly impair pancreatic B – cell function⁸. It has been suggested that the hyperglycemia associated with protease inhibitor therapy maybe as a result of the inhibition of the protease that converts proinsulin to insulin¹⁶.

V. Conclusion

The use of protease inhibitor regimen may predispose HIV patients to diabetes mellitus (DM). The result of this study calls for attention and it is advised that HIV patients on protease inhibitors regimen be monitored every 3 months to screen for the development of hyperglycaemia. Blood sugar levels should be checked before the onset of treatment. This will identify subjects who are at greater risk, so that alternative drugs will be used for their treatment. Patients who have a family history of diabetes should be placed on alternative drugs. It is further recommended that protease inhibitors (PIS), unless absolutely necessary be replaced with other equally potent classes of antiretrovirals. Further studies of longer duration will be helpful in determining the longer term effects of these protease inhibitors.

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