ASSESSMENT OF FASTING PLASMA GLUCOSE, INSULIN, INSULIN RESISTANCE, INTERLEUKIN 10, HIGH SENSITIVE C - REACTIVE PROTEIN AND TUMOUR NECROSIS FACTOR ALPHA IN HIV INFECTED ADULTS WITH AND WITHOUT DIABETES MELLITUS IN NAUTH, NNEWI.

ABSTRACT

Background and Aim of Study: To assess the levels of fasting plasma glucose, insulin, insulin resistance, interleukin 10 (lL-10), high sensitive C - reactive protein (Hs-Crp) and tumour necrosis factor alpha (TNF-α) in HIV infected adult individuals with and without diabetes mellitus in NAUTH Nnewi, Nigeria.

Study Design: This was a cross-sectional study design.

Place and Duration of study: HIV clinic, Nnamdi Azikiwe university teaching hospital,Nnewi Nigeria.

Methodology : A total of 150 participants were recruited using simple random sampling technique and comprised 50 HIV infected individuals with diabetes mellitus, 50 HIV infected individuals without DM and 50 apparently healthy participants without HIV nor DM (control). TNF-α, IL-10 and Hs-Crp was determined using ELISA technique. Anthropometric parameters and blood pressure were measured using standard laboratory methods.

Results: There was significantly higher mean level of TNF-alpha, fasting insulin, HOMA-IR, Hs-Crp and FPG in HIV infected individuals with and without DM when compared with control group ($p<0.05$) respectively. Significantly higher mean value of BMI was observed in HIV infected individuals with DM compared with HIV infected individuals without DM ($p<0.05$).BP was significantly higher in HIV infected individuals with DM compared with their counterpart without DM and control ($P<0.05$) respectively. However, the mean IL-10 level did not differ significantly ($P>0.05$) between the groups studied.

Conclusion: This suggests some degree of inflammatory reaction in HIV infected individuals without DM and this may subsequently predispose the affected individuals to type 2 DM.

Key words: HIV, Type 2 diabetes mellitus, interleukin 10, high sensitive C - reactive protein, tumour necrosis factor alpha.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic condition caused by decreased or absent insulin production or possibly by decreased tissue sensitivity to insulin.[1,2] According to recent studies, the disease is currently on the rise in Nigeria with a prevalence of 5.77% among Nigerians[3].Type 2 diabetes mellitus (T2DM) is believed to be associated with low-grade chronic inflammation.[4]Both HIV and diabetes mellitus seems to be associated with inflammation which results from dsyregulation in cytokine release.

Changes in cytokine levels in HIV infected individuals can affect the function of the immune system and have the potential to directly impact the course of HIV disease by enhancing or suppressing HIV replication.[5]Most of these cytokines have been used as hallmarks of disease progression as well as assessment of patient's response to antiretroviral treatment.[6]It has been previously documented that HIV replication is the result of a balance between the effects of pro-inflammatory cytokines that increase viral replication and those of anti-inflammatory cytokines and chemokines that inhibit viral replication.[7] With the growing understanding of their roles during infections and disease progression, cytokines including IFN-γ, IL-10 and tumor necrosis factor alpha (TNF-α) have been assayed in plasma to assess the efficacy of antiretroviral therapy during HIV infection.[8] For example, HAART markedly increases plasma IFN-γ levels.[9]and considerably lowers IL-10 systemic levels during HIV infection.[10] Hence, these actions co-operatively show that antiretroviral treatment markedly influences systemic cytokine levels. Chronic immune activation and inflammation are consistently found in PLWH and T2D is also characterized by similar findings.[11]Low grade systemic inflammation has been hypothesized as an underlying factor for the pathogenesis of T2D[12-14]when there is elevated plasma levels of C-reactive protein (CRP) and IL-6.[12]CRP, an acute phase protein is synthesized by hepatocytes in response to pro-inflammatory cytokines, in particular IL-6.[15]TNF- alpha is a cytokine that promotes inflammation[16], and has been linked to the development of insulin resistance, which aids in the development of type 2 diabetes.[17]Ingle and Patel reported that hyperglycaemia stimulates the release of the inflammatory cytokines, tumour necrosis factor (TNF-α) and IL-6 from various cell types and can also result in the induction and secretion of acute phase reactants by adipocytes.[18]

C-reactive protein (CRP), a positive acute phase reactant (protein), was first detected in the bloodstream in response to hepatocyte inflammation.[19]It is a marker of inflammation which characterizes several chronic disorders. One biochemical test that is very sensitive in quantifying CRP is the high-sensitivity C-reactive protein test (Hs-CRP). The newly improved assay known as "high sensitivity" detects incredibly low plasma levels of CRP.[19]Giving us a general notion of the inflammatory status, it estimates the general amounts of inflammation in the human body.[19]

IL-10 on the hand has powerful anti-inflammatory properties and plays a significant role in limiting an excessive inflammatory response.[20]The immune system relies on IL-10 to maintain the proper balance in the immunological response required to defend the body and safeguard tissues from infection.[21]

Plasma glucose is a metabolic fuel of the body, excess of which gives rise to hyperglycemia which characterizes diabetes mellitus.[1]

Insulin is a polypeptide hormone that controls blood glucose levels and is mostly secreted by beta cells in the pancreatic islets of Langerhans.[22]The onset and progression of certain chronic diseases are also influenced by insulin production and levels.[22]

Insulin resistance (IR) is a common medical condition or state where normal responses require higher than usual insulin concentrations, which subsequently results in hyperinsulinemia and impaired insulin action in certain cases.[23]Insulin resistance affects several organs, including adipose tissue, muscle, and the liver, and impairs insulin signaling pathways.[24]A person with homeostatic model assessment index (HOMA-IR) ≥ 2.5 is considered to be insulin resistant.[23]

It is widely acknowledged that insulin resistance, which exacerbates dyslipidemia and dysregulated glucose metabolism, is correlated with activation of the innate immune system.[25] Patients receiving continuous antiretroviral therapy (cART) and untreated HIV patients have both been shown to experience varying degrees of chronic inflammation as a result of immune activation.[26]Nonetheless, untreated HIV patients exhibit a heightened inflammatory state that is linked to a procoagulant condition and is marked by elevated levels of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukins (IL-6 and IL-1β).[27] Insulin resistance is likely significant under these circumstances and may affect adipose, muscle, and liver tissue. Therefore, the current study is focused on determining the levels of fasting plasma glucose, insulin, insulin resistance, lL-10, Hs-Crp and TNF-αin HIV infected adult individuals with and without diabetes mellitus in NAUTH Nnewi, Nigeria.

MATERIALS AND METHODS

Study Design

This research was a cross-sectional study designed to assess the levels of fasting plasma glucose, insulin, insulin resistance, lL-10, Hs-Crp and TNF-α in HIV infected adult individuals with and without diabetes mellitus in NAUTH Nnewi, Nigeria. A total number of 150 subjects categorized into 3 groups were recruited for the study using simple sampling technique. Group 1 comprised 50 HIV infected adult individual with diabetes mellitus, Group 2 comprised 50 HIV infected adult individuals without diabetes mellitus while group 3 comprised 50 apparently healthy controls that are neither diabetic nor HIV positive.

The test subjects (Groups 1 and 2) were selected from the NAUTH HIV clinic. They were both male and female and ranged in age from 18 to 75. The control group comprised apparently healthy individuals between the ages of 28 to 53 years and was selected from the staff and

students of the Nnamdi Azikiwe University Teaching Hospital Nnewi.Information on socio-demographic, medical history and life styles were obtained using standard questionnaire. Also, anthropometric parameters and blood pressure were determined using standard methods. All HIV infected individuals were on antiretroviral therapy.

Ethical Approval

Ethical approval for this study was obtained from the board of ethics committee ofNnamdi Azikiwe University Teaching Hospital, Nnewi (NAUTH/CS/66/VOL.13/VER.3/274/2021/057).

Informed Consent

Before commencement, informed consent of all the participants wasobtained before enrolment into the study.The participants were assured of confidentiality of the information obtained from them during and after the study.

Inclusion Criteria

According to CDC and WHO HIV disease staging,only HIV stage II subjects and subjects who had commenced anti-retroviral therapy were recruited. Additionally, test participants between the ages of 18 and 75 and control subjects (apparently healthy) between the ages of 28 and 53 were selected for this study.

Exclusion Criteria

This study excluded the following groups of people: pregnant women, HIV-positive people not receiving antiretroviral therapy (ART), people in Stage 3 of the infection, people with tuberculosis, people infected with malaria, people in the age range of 10 to 17 years, people with tumors or any inflammatory disease, and people with chronic illnesses other than HIV.

Blood Sample Collection and Processing

Six milliliters (6 ml) of venous fasting blood samples were collected aseptically after 10-12 hours of fast by venipuncture from each subject via the antecubital vein using a plastic syringe with minimum stasis into fluoride oxalate and plain containers. 2 ml of venous blood was dispensed into fluoride oxalate for determination of fasting plasma glucose (FPG) level which was assayed immediately without storage. The remaining 4 ml of the venous blood sample was dispensed into plain container and allowed to clot and retracted. It was then centrifuged at 4000

rpm for 5 minutes and serum separated and used for analysis of biochemical markers. Serum samples that were not analyzed immediately were stored frozen at -20°C.

Laboratory Methods

Determination of Fasting Plasma Glucose Concentration

Fasting Plasma glucose were determined by enzymatic method as described by Frank *et al*. [28]

Determination of Fasting Insulin Using Enzyme Linked Immunosorbent Assay.

Human insulin was assayed using the sandwich enzyme linked immunosorbent assay (ELISA) as described by Burgi *et al*.[29]

Determination of insulin resistance index

The insulin resistance index of each subject was determined by Homeostatic model assessment (HOMA) according to the method described by Hashemipour *et al*.[30]The insulin resistance score was computed with the formula: Fasting plasma glucose (mmol/L) times fasting serum insulin (mU/L) divided by 22.5.

Determination of Human Il-10(Interlukin 10) by Enzyme linked immunosorbent assay.

Human IL-10 level was assayed using Sandwich Enzyme linked immunosorbent assay(ELISA)technique as described by Chiswick *et al.*[31]

Determination of High Sensitivity C-Reactive Protein Enzyme Immunoassay Test

High sensitive C-reactive protein was assayed using the solid phase enzyme linked immunosorbent assay(ELISA) technique as described by (Votruba and Rakosnik.[32]

Determination of Tumour necrosis factor-alpha

Tumour necrosis factor-alpha was assayed using the sandwichEnzyme immunosorbent linked method(ELISA) as described by Engelberts*et al*.[33]

Anthropometric measurement

The weight and height of each participant were measured using a standard beam balance scale and a stadiometer respectively.Body mass index (BMI) was calculated as weight (kg) divided by height squared in meters.

$BMI(Kg/m^{2)} = Weight(Kg)/Height^2\ (m^{2).}$

Also, systolic blood pressure (SBP) and diastolic blood pressure (DBP) was measured using sphygmomanometer and stethoscope.

Statistical analysis

Statistical package for social sciences version 23.0 was used for data analysis.The data obtained were analyzed using ANOVA, Posthoc t-test and Pearson Correlation. Results were deemed significant at $p < 0.05$.

RESULTS

The result of analysis of variance (ANOVA) showed that the mean serum levels of TNF-α, fasting insulin, HOMA-IR, Hs-Crp and fasting plasma glucose were significantly different amongst the group (F=11.880, 16.222, 39.340, 7.624 and 8.312) (P<0.05) respectively, whereas, IL-10 did not differ significantly amongst the group (F=1.388; P>0.05) (See table 1).

The mean (±SD) of serum TNF-α (pg/L) level in the HIV seropositive individuals with diabetes mellitus (15.82±2.59) was significantly higher compared with the corresponding values in the HIV seropositive individuals without diabetes mellitus (10.46±2.54) and control group (5.46±2.32) respectively (p=0.036; 0.024). Also, the mean serum TNF-α level was significantly higher in the HIV seropositive individuals without diabetes mellitus compared with control group (10.46±2.54 Vs 5.46±2.32; p=0.030). See table 1.

Also the mean serum IL-10 (pg/L) level in the HIV seropositive individuals with diabetes mellitus did not differ significantly compared with the HIV seropositive individuals without diabetes mellitus and control group (p>0.05). There was no significant difference in the mean serum IL-10 level in the HIV seropositive individuals without diabetes mellitus compared with control group (p=0.352). See table 1.

There was significantly higher mean serum fasting insulin (ng/ml) level in the HIV seropositive individuals with diabetes mellitus compared with HIV seropositive individuals without diabetes mellitus (24.79±7.69 Vs 13.10±3.35; p=0.000). Also, there was significantly higher mean serum level of fasting insulin observed in the HIV seropositive individuals with diabetes mellitus compared with control group (24.79±7.69 Vs 7.12±3.50; p=0.000). The mean serum insulin level wasalso significantly higher in the HIV seropositive individuals without diabetes mellitus compared with control group (13.10±3.35 Vs 7.12±3.50; p=higher0.028). See table 1.

The mean (±SD) of HOMA-IR levels in the HIV seropositive participants with diabetes mellitus was significantly compared with the corresponding values in the HIV seropositive participants

without diabetes mellitus and control group (p=0.000; 0.001) respectively. Also, the mean (±SD) of HOMA-IR level in the HIV seropositive participants without diabetes mellitus was significantly higher compared with the observed value in the control subjects (3.77±2.76 Vs 0.31±1.66; p=0.036). See table 1.

There were significantly higher mean serum levels of Hs-Crp mg/dl) in the HIV seropositive participants with diabetes mellitus compared with those without diabetes mellitus and control subjects (p=0.023; 0.006) respectively. Also, the mean (±SD) of serum Hs-Crp level was significantly higher in the HIV seropositive participants without diabetes mellitus than in the control group (6.16±1.33Vs 2.28±1.35; p=0.041). See table 1.

Furthermore, the mean plasma fasting glucose was significantly higher in the HIV seropositive participants with diabetes mellitus compared with those without diabetes mellitus (10.24±2.82 Vs 5.90±0.56; p=0.031). It was also significantly higher in the HIV seropositive participants with diabetes mellitus compared with control group (10.24±2.82 Vs 4.17±0.67; p=0.001). Furthermore, it was also significantly higher in the HIV seropositive participants without diabetes mellitus than in control group (5.90±0.56 Vs 4.17±0.67; p=0.047). See table 1.

The result of analysis of variance (ANOVA) showed that the mean levels of body mass index (kg/m^2), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) were significantly different amongst the group (F=4.065, 15.832 and 9.501) (P<0.05) respectively, whereas, the mean age (years) did not differ significantly amongst the group (F=0.338; p=0.714) (See table 1).

The mean body mass index (BMI) was significantly higher in the HIV seropositive participants with diabetes mellitus compared with those without diabetes mellitus (29.45±6.28 Vs 27.19±5.17; p=0.028), but it did not differ significantly when compared between the HIV seropositive participants with diabetes mellitus and control group (p=0.232) as well as between those without diabetes mellitus and control group (p=0.897). See table 2.

There was significantly higher mean systolic blood pressure in the HIV seropositive participants with diabetes mellitus compared with those without diabetes mellitus (p=0.000) and control subjects (p=0.000) respectively but it did not differ significantly when compared between the HIV seropositive participants without diabetes mellitus and control group (p=0.462). See table 2.

There was significantly higher mean diastolic blood pressure in the HIV seropositive participants with diabetes mellitus compared with those without diabetes mellitus (p=0.000). It was also

significantly higher in the HIV seropositive participants with diabetes mellitus compared with control subjects (p=0.000) but it did not differ significantly when compared between the HIV seropositive participants without diabetes mellitus and control group (p=0.078). See table 2.

There were no statistically significant correlation observed between the levels of TNF-α Vs fasting insulin, FPG and HOMA-IR in the HIV infected individuals with diabetes mellitus (p>0.05) respectively. See table 3.

There were no statistically significant correlation observed between the levels of IL-10 Vs fasting insulin and HOMA-IR (p>0.05), but there was a significant negative correlation seen between IL-10 and FPG levels in the HIV infected individuals with diabetes mellitus (r=-0.482; p=0.011). See table 3.

Also, significant positive correlations were observed between the levels of Hs-Crp and fasting insulin (r=0.473; p=0.013) and between Hs-Crp Vs FPG (r=0.401; p=0.038) although no significant correlation was observed between the levels of Hs-Crp and HOMA-IR in the HIV infected individuals with diabetes mellitus (p=0.576). See table 3.

There was no significant correlation observed between the parameters studied in the infected individuals without diabetes mellitus (p>0.05). See table 4.

Furthermore, no significant correlation was observed between the parameters studied in the control group (p>0.05). See table 5.

Table 1: Levels of TNF-α, IL-10, fasting insulin, HOMA-IR, Hs-Crp and FPG in HIV infected adult individuals with diabetes mellitus, HIV infected adult individuals without diabetes mellitus and control (Mean ±SD)

Group	TNF-α (pg/L)	IL-10 (pg/L)	Fasting insulin (ng/ml)	HOMA-IR	Hs-Crp (mg/dl)	FPG (mmol/L)
HIV WITH DM(A)	15.82±2.59	112.44±31.6	24.79±7.69	9.43±6.59	10.62±6.80	10.24±2.82

HIV W/O DM (B)	10.46±2.54	98.48±30.41	13.10±3.35	3.77±2.76	6.16±1.33	5.90±0.56
Control(C)	5.46±2.32	119.95±29.22	7.12±3.50	0.31±1.66	2.28±1.35	4.17±0.67
F-test	11.880	1.388	16.222	39.34	7.624	8.312
P- value	0.014*	0.255	0.001*	0.000*	0.000	0.020
A vs B	0.036*	1.000	0.000*	0.001*	0.023*	0.031
A vs C	0.024*	0.657	0.000*	0.000*	0.006*	0.001
B vs C	0.030	0.352	0.028*	0.036	0.041	0.047

*Statistically significant at $p<0.05$.

TNF-α= Tumour necrosis factor-alpha, IL-10=Interlukin-10, Hs-Crp=High sensitive C-reactive protein, HOMA-IR= Homeostasis model assessment index, FPG= Fasting plasma glucose.

Table 2: Age, BMI, Systolic blood pressure (SBP) and diastolic blood pressure (DBP) in HIV infected adult individuals with diabetes mellitus, HIV infected adult individuals without diabetes mellitus and control (Mean ±SD)

Group	Age (Yrs)	Weight (Kg)	Height (m)	BMI (Kg/m^2)	SBP (mmHg)	DBP (mmHg)
HIV WITH DM (A)	37.89±4.36	88.04±14.21	1.74±0.10	29.45±6.28	125.96±11.95	72.16±10.00

HIV W/O DM (B)	39.03±7.14	76.86±13.83	1.68±0.05	27.19±5.17	108.07±8.98	65.86±6.52
Control (C)	39.84±12.81	82.48±11.25	1.69±0.08	27.15±5.16	112.65±15.02	71.55±6.35
F-test	0.338	5.096	3.850	4.065	15.832	9.501
P-value	0.714	0.008	0.025	0.024	0.000	0.000
A vs B	1.000	0.006	0.029	0.028	0.000	0.000
A vs C	1.000	0.333	0.117	0.232	0.000	0.000
B vs C	1.000	0.301	1.000	0.897	0.462	0.078

*Statistically significant at $p<0.05$.

Table 3: Correlation of TNF-α, IL-10, Hs-Crp, with Fasting insulin, HOMA-IR and FPG in HIV infected adult individuals with diabetes mellitus.

Parameters	**Fasting insulin** (Ng/ml)	**FPG** (Mmol/L)	**HOMA-IR**
TNF-α (Pg/L) **r-value**	0.043	0.123	0.291
P value	0.830	0.542	0.141

IL-10 r-value	0.108	-0.482	0.090
(Pg/L) **P value**	0.591	0.011*	0.654
Hs-Crp r-value	0.473	0.401	0.113
P value	0.013*	0.038*	0.576

*Statistically significant at $p<0.05$.

Key:

TNF-α= Tumour necrosis factor-alpha, IL-10=Interlukin-10, Hs-Crp=High sensitive C-reactive protein, HOMA-IR= Homeostasis model assessment index, FPG= Fasting plasma glucose.

Table 4: Correlation of TNF-α , IL-10, Hs-Crp with fasting insulin, HOMA-IR and FPG in HIV infected adult individuals without diabetes mellitus.

Parameters	**Fasting insulin**	**HOMA-IR**	**FPG**
TNF-α r-value	0.173	0.289	0.209
P value	0.929	0.129	0.287
IL-10 r-value	0.018	0.154	0.148
P value	0.926	0.424	0.452

Hs-Crp r-value	0.027	0.072	0.303
P value	0.891	0.709	0.117

*Statistically significant at $p<0.05$.

Key:

TNF-α= Tumour necrosis factor-alpha, IL-10=Interlukin-10, Hs-Crp=High sensitive C-reactive protein, HOMA-IR= Homeostasis model assessment index, FPG= Fasting plasma glucose.

Table 5: Correlation between TNF-α, IL-10, Hs-Crp, with fasting insulin, HOMA-IR and FPG in control group.

Parameters	**Fasting insulin**	**HOMA-IR**	**FPG**
TNF-α r-value	0.082	0.099	0.084
P-value	0.659	0.597	0.653
IL-10 r-value	-0.082	0.910	0.073
P value	0.659	0.654	0.659

Hs-Crp r-value	0.208	0.031	0.261
P value	0.262	0.867	0.156

*Statistically significant at $p<0.05$.

Key:

TNF-α= Tumour necrosis factor-alpha, IL-10=Interlukin-10, Hs-Crp=High sensitive C-reactive protein, HOMA-IR= Homeostasis model assessment index, FPG= Fasting plasma glucose.

DISCUSSION

The results of the current investigation demonstrated that, in comparison to HIV-positive individuals without diabetes mellitus, those with diabetes had a significantly higher mean level of FPG. Furthermore, compared to controls, adult HIV-positive persons with and without diabetes mellitus showed significantly higher mean levels of FPG. This is in keeping with previous similar research that shows increased blood glucose in diabetic people with and without HIV infection.[34]The source of the higher plasma glucose levels may be due to the effects of antiretroviral medication, HIV infection, or both, which worsened the glucose metabolism in this individuals.[35,36]According to Chukwuanukwu*et al*., FPG levels were likewise considerably higher in the non-diabetic HIV seropositive group than in the non-diabetic HIV seronegative group[37], which is consistent with the findings of the current investigation.

The study findings showed that HIV-positive people with diabetes mellitus had a significantly higher mean serum level of fasting insulin than HIV-positive adults without diabetes mellitus or control subjects. This study also found a significantly higher mean serum insulin level in the HIV-positive individuals without diabetes mellitus as compared to the control group. The higher insulin levels observed in the HIV infected individuals without diabetes mellitus in this study may be an indication that these individuals are at risk of developing type diabetes mellitus as a result of insulin resistance orchestrated by HIV infection and due to the effect of antiretroviral drugs which promotes chronic inflammatory immune responses. Potential mechanisms associated with diabetes in HIV infected persons include HIV infection itself, use of antiretroviral drugs, insulin resistance and immune activation which induces inflammatory

responses leading to the release of heightened levels of inflammatory cytokines.[38]In the event of prevailing hyperglycemia, insulin is secreted from the beta cells of the islets of Langerhans in the pancreas to enhance the uptake of the excess glucose into insulin-sensitive tissues, such as the liver, muscle, or adipose tissue. Insulin is a key hormone in the regulation of plasma glucose levels in the human body. Therefore, in cases of persistently high blood sugar, insulin-sensitive tissues may become resistant to the effects of insulin and lose their sensitivity. In response, the pancreas secretes more insulin than usual in an attempt to restore a normal metabolic response.

In this study, the mean insulin resistance as determined by homeostasis model assessment index (HOMA-IR) was significantly higher in the HIV seropositive individuals with and without diabetes mellitus than in control group respectively. This finding suggests that insulin resistance may be the main driver of diabetes mellitus among HIV-positive adults.[39]Insulin resistance in HIV infected patients could be due to immune activation whether the patient is on HAART or not.[40] This immune activation has been observed in HIV infected patients undergoing therapy and those not undergoing therapy.[26] Several studies have reported varying degree of prevailing insulin resistance in HIV infected with and without diabetes mellitus and this is in consonance with the present report.[41,42]

In this study,there was no significant difference in the mean level of IL-10 in HIV infected adult individuals with diabetes mellitus, HIV infected adults individuals without diabetes mellitus and control group.This could be due to highly antiretroviral therapy which suppresses viral replication at different stages of HIV life cycle, improves immunological status of HIV infected individuals and delays drug resistance.[43,44] This is in keeping with the report of previous similar study that observed no significant difference in IL-10 level in HIV infected individuals compared to control participants after 12 months of antiretroviral therapy.[6]

This study found significantly higher mean serum level of Hs-Crp in HIV infectedindividuals with diabetes mellitus comparedwith HIV infected individuals without diabetes mellitus and control group. This in line with reports of previous study which showed a significant increase in hs-CRP levels in people living with HIV (PLWH) on HAART when compared to negative control.[45] Furthermore, in keeping with the current finding, Chukwuanukwu et al. in their study reported significantly higher mean serum C-reactive protein levels in Diabetic HIV seropositive subjects and non diabetic HIV seropositive subjects compared to control subjects.[37]This may suggest that chronic inflammation in HIV seropositive individuals can predispose them to

development of diabetes mellitus. An increased CRP level can be caused by both acute inflammation (which may then decrease) and unresolved persistent inflammation. Both resolved and unresolved inflammation can be identified and measured using high-sensitivity C-reactive protein.[46] One of the main long-term problems for HIV patients is cardiovascular disease (CVD), and Hs-CRP is thought to be a possible biomarker for predicting CVD risk and long-term disease progression.[46]

In this investigation, the mean serum level of TNF-α was shown to be significantly higher in HIV-positive adults with diabetes mellitus than in HIV-positive adults without diabetes mellitus or control subjects. Also, there was significantly higher mean serum TNF-α level in the HIV infected individuals without diabetes mellitus than in control group. This shows that both HIV infection as well as diabetes mellitus is characterized by inflammation which potentiates insulin resistance that may culminate in diabetes mellitus. Persistent cytokine activation leading to prolonged release of TNF-α and other inflammatory cytokines can lead to development of insulin resistance which can result in the development of diabetes mellitus.[39]

This report agrees with the finding of Osuji et al. which showed significantly higher mean serum TNF-α level in HIV infected persons even after 12 months antiretroviral therapy.[6] This phenomenon may be present even in the absence of exposure to antiretroviral therapy (ARV) medications typically associated with insulin resistance risk.

The present study showed the HIV-positive individuals with diabetes mellitus had a mean body mass index that was significantly higher than that of those without diabetes mellitus. Notably, when comparing HIV-positive people with and without diabetes mellitus to control subjects, no significant changes in their mean BMI were seen. Remarkably, according to the World Health Organization's classification of BMI, which is provided below, both the test individuals and the control group in this study were overweight: Below 18.5 kg/m^2, normal weight = 18.5 - 24.9 kg/m^2, overweight = 25.0 - 29.9 kg/m^2, obesity class I = 30.0 - 34.9 kg/m^2, obesity class II = 35.0 -39.9 kg/m^2, and obesity class III $\geq$ 40.0 kg/m^2.[47] Being overweight raises insulin resistance, which can accelerate the development of a number of chronic diseases for which it is a significant risk factor. Ezeugwunne *et al*., in keeping with the present findings, found no statistically significant changes in the mean BMI between HIV-positive subjects on antiretroviral therapy (ART) and control subjects.[48].

Also, a significantly higher mean value was observed in SBP in HIV infected adult individuals without diabetes mellitus and control. According to a prior cross-sectional study investigating the effect of HAART on hypertension in HIV-positive people residing in Cameroon's North West region (NWR), 29.84% of the participants had systemic hypertension.[49] This is consistent with current findings.

IL-10 and FPG levels were found to be significantly inversely correlated in HIV-positive persons with diabetes mellitus. Additionally, among HIV-positive persons with diabetes mellitus, there were noteworthy positive associations found between Hs-Crp levels and fasting insulin as well as between Hs-Crp and FPG.

Conclusion

This study showed higher levels of TNF-alpha, fasting insulin, HOMA-IR, Hs-Crp and FPG in the HIV seropositive individuals with and without diabetes mellitus compared to the control group. This is suggestive of ongoing inflammatory reactions which could subsequently lead to development of insulin resistance (IR), thereby causing or predisposing the HIV infected individuals at risk of developing type 2 diabetes mellitus.

Consent: All authors declare that 'Written informed consent" was obtained from the patient for the publication of this research. A copy of written consent is available for review by editoral office/chief editor/editorial board members of this journal.

Ethical approval: All authors hereby declare that the experiments have been examinedand approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

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APPENDIX

INFORMED CONSENT FORM

Affiliation Of research

I am OBILO CHIDERA VIVIENNE, a postgraduate student of the department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus. I am under supervision, carrying out a research on “Assessment Of Pancreatic Functions and Some Inflammatory Markers In Hiv infected Individual in NAUTH, Nnewi, Nigeria ”.

Purpose Of research

The research is a pre-requisite for an award of Master Of Science Degree In Medical Laboratory Science.

Aim of the study

This study aims to assess the pancreatic function and some inflammatory markers in Hiv infected individuals in NAUTH, Nnewi, Nigeria.

Confidentiality of information.

The confidentiality of all participants in the study will be ensured.Information obtained from subjects will not be divulged to anyone unrelated to this study.No name will be recorded as information about participants will be stored with code numbers.

Requirement from each participant.

Five (5) millilitres of blood will be taken via venepuncture using syringe and needle.

Voluntarism

Participation in this study is entirely voluntary

Participants can choose to withdraw from this research anytime

Risk/Benefits

This study will not expose a participant to any form of hazard.The benfits are that your results can be revealed to you and proper advice granted through the available facilities.

Cost

Participants will neither be paid nor charged.

Statement of person obtaining information consent: I have fully explained this research to volunteers.I have given sufficient information including risk and benefits to enable the participants make an informed decisions.

Date..... Signature.....

Name..... Phone No.....

Statement of person giving consent: I have read the description of the research and understood that any participation and withdrawal is voluntary.I have also been well informed of the risk benefits to judge that i want to participate in it.

Date..... Signature.....

Name..... Phone.....

NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL

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Our Ref:

Your Ref:

15th September, 2021

Date:

Obilo Chidera Vivienne,
Department of Medical Laboratory Science,
Faculty of Health Science and Technology,
College of Health Sciences,
Nnamdi Azikiwe University,,
Awka

ATTENTION: NOTICE OF FULL NAUTH HEALTH RESEARCH ETHICS COMMITTEE APPROVAL

RE: ASSESSMENT OF PANCREATIC FUNCTIONS AND SOME INFLAMMATORY MARKERS IN DIABETIC HIV INDIVIDUALS IN NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI

This is to inform you that the research described in the submitted protocol, the consent form and other participant's information materials have been reviewed and full approval granted by NAUTHHREC.

The approval is for one year starting from 15th September, 2021 to 14th September, 2022. If there is delay in starting the research, please inform the Secretariat so that the date of the approval will be adjusted accordingly.

In multiyear research, you are required to submit your annual report to Secretariat early in order to renew your approval and avoid disruption of the research.

You are not permitted to make changes in the research without prior notification and approval of the NAUTHHREC.

We reserve the right to conduct compliance visit to your research site without previous notification.

Please note that this approval is subject to revocation, if you fail to adhere to these guidelines.

Mrs. Rita C. Ikeanyionwu
Sec., NAUTHHREC

Prof. Chisolum Okafor
Chairman, NAUTHHREC