Elite Journal of Health Sciences. Volume 2 issue 2(2024), Pp. 30-39
https://epjournals.com/journals/EJHS

Assessment of Serum Cystatin C, Microalbumin Levels and EGFR in HIV Seropositive Individuals in NAUTH, Nnewi, Nigeria

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Abstract

Human immunodeficiency virus (HIV) is now a confirmed risk factor for kidney disease with higher burden in persons of African origin. The aim of this study is to assess the renal function of HIV seropositive patients in NAUTH Nnewi using Cystatin C, Microalbuminuria and eGFR as biomarkers. This study was a cross-sectional study in which simple random sampling technique was employed in the selection of eighty-two (82) study participants within the age of 18yrs and above, divided into two (2) groups: Test group - consist of forty-two (42) HIV-seropositive patients, and Control group - constitutes forty-two (42) apparently healthy HIV seronegative individuals. In this study, questionnaires were used to obtain vital information such as, socio demographic data, the medical and health information from the participants after consent had been obtained. Blood and urine samples were collected while Cystatin C, Microalbumin and eGFR level determined via a known standard method. The result shows no significant difference in the levels of cystatin C and eGFR in the test HIV-positive patients when compared to their control

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counterparts. However, we observed a significantly higher levels of microalbumin in the test group when compared to the control. In conclusion, the findings of this study suggests that there is significantly elevated microalbumin levels among patients with HIV when compared to the control group indicating the presence of renal damage or renal problem, suggesting the presence of HIV-induced kidney diseases in the test group.

Keywords: *Cystatin C, Microalbumin Levels and EGFR in HIV*

Introduction

Human immunodeficiency virus (HIV) is a blood-borne, sexually transmissible virus. The virus is typically transmitted via sexual intercourse, shared intravenous drug paraphernalia, and mother-to-child transmission (MTCT), which can occur during the birth process or during breastfeeding. The most common route of infection varies from country to country and even among cities, reflecting the population in which HIV was introduced initially and local practices. Co-infection with other viruses that share similar routes of transmission, such as hepatitis B, hepatitis C, and human herpes virus 8 (HHV8; also known as Kaposi sarcoma herpes virus [KSHV]), is common.[1-7]

Cystatin C (CysC), a non-glycosylated protein, is a biomarker of glomerular filtration. CysC is a small molecule, 13 kDa in size, that is filtered from the blood through the glomerulus and catabolized, but not secreted, by the proximal tubular cells, and is produced by all nucleated cells at a constant rate. It is a member of the family of cysteine proteinase inhibitor that has gained popularity in the measurement of renal function and determination of the estimated glomerular filtration rate (eGFR).[8-9]

Cystatin C has been suggested as a potential alternative to serum creatinine, as it potentially has fewer non-GFR determinants. In epidemiological studies, early stages of kidney function decline can be detected more readily by eGFR based on cystatin C thus offering the opportunity to identify chronic kidney disease (CKD) earlier than when using creatinine-based eGFR. This improved approximation of GFR across the higher end of kidney function has resulted in cystatin C having far stronger associations than creatinine with long-term cardiovascular outcomes in numerous population-based cohort studies. Over the past 15 years, CysC's role has been relegated to its being an outstanding research tool.[8-9]

MATERIALS AND METHODS

Methods

Study Site

The participants for this study were recruited from the clinic of Institute of Human Virology Nigeria (I.H.V.N) Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria.

Study Design

This is a cross-sectional study that was designed to evaluate the serum levels of Cystatin C, Microalbuminuria and eGFR in HIV seropositive outpatients attending I.H.V.N clinic in Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra State, Nigeria. HIV seropositive patients were selected by random sampling from the I.H.V.N clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH). Forty-two (42) control group subjects (27 females and 15 males) were randomly selected. A total of Forty-two (42) HIV seropositive (test) subjects (27 females and

15 males) were also recruited for this study. Structured questionnaire was administered to each participant to collect their bio-data and other medical records. HIV seropositive subjects within the range of 20-50 years was selected irrespective of their gender and ethnicity.

Calculation of Sample Size

The sample size was calculated using the method described by Charan and Biswas (2013);

$$N = \frac{(Z^2pq)}{d^2}$$

Where:

N= Desired number of sample when population of the facility is limited

Z = Z value, where Z is the standard normal variance where confidence level is 1.96 at 95%
p = Prevalence rate of HIV in Nigeria which is 2.8% (National Agency for the Control of HIV/AIDS (NACA), 2017).

q = 1 – p

d = 5% i.e., degree of precision as desired by the researcher.

Applying the method,

$$N = \frac{Z^2 \times p \times (1-p)}{d^2}$$

$$N = \frac{1.96^2 \times 0.028x (1-0.028)}{0.05^2}$$

$N = 41.82 \approx 42$

Inclusion Criteria.

HIV seropositive patients attending IHVN Clinic at NAUTH, who were already on ART drugs were included in the study. The control subjects were apparently healthy individual that gave their informed consent. All subjects were ≥ 18 years but ≤ 50 years of age.

Exclusion Criteria

Subjects that are taking drugs which are known to affect any of the parameters to be evaluated, smokers, individuals with known disorders such as; liver diseases, vascular disorders, who have received kidney transplant, HIV/ Tuberculosis patients, HIV/ Hepatitis B & or C patients, as well as any other physical illness, < 18 years or > 50 years of age or failed to obtain a written consent, were excluded from this study.

Ethical Consideration

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The ethical approval for this research was obtained from Nnamdi Azikiwe University Teaching Hospital ethics committee in accordance with the Helsinki declaration by the World Medical Association (WMA) on the ethical principles for medical research involving human subjects.

Collection of samples

10mls of blood sample was collected in total from each subject via the ante cubital vein. Rubber tourniquet was applied for less than one minute and the site to be punctured was cleaned with an alcohol swab and the blood was collected using a vacutainer. 3mls of blood was added into both the fluoride oxalate container and the plain container. The blood in the plain vacutainer was allowed to clot in an upright position for at least 30 minutes.

Centrifugation of the blood lasted for at least 15 minutes at 3000 RPM and serum was separated, transferred to a plain (red capped) sample container. The samples were stored at -20 ºC until analysis. Five (5) mL of first early morning urine was collected before breakfast or exercise in a universal container. The plain container which contains the collected urine sample was stored at a temperature of 8-10 °c until analysis.

Laboratory Analysis

Estimation of Cystatin C

The Cystatin C Assay is based on a latex enhanced immunoturbidimetric assay. Cystatin C in the sample binds to the specific anti Cystatin C antibodies, which are coated on latex particles and causes agglutination.

Assay Procedure

3µl of serum and 3µl of calibrator was dispensed into the microplate wells, respectively. 180µl of reagent R1 ((Tris buffer, 20 mmol/L, pH 8.3, sodium azide; 0.95 g/L) was dispensed into each microplate well, mixed and was incubated for five (5) minutes at 37^0C. Afterwards, the absorbance (A1) of was read at 570 nm using Biobase microplate reader.

Immediately, 60µl of Reagent R2 (CYS-C antibody latex particle, tris buffer, 50 mmol/L, pH 7.5, sodium azide 0.95 g/L) were dispensed into each of the microplate well, mixed and was read again at 570nm after 5 minutes of incubation at 37^0C as A2 for calibrator and sample respectively.

Estimation of Microalbumin

Method: Immunoturbidimetric

Assay Procedure

15µl of participants' urine samples and 15µl of calibrator were dispensed into the microplate wells, respectively. 250 µl of reagent R1 ((Tris buffer, 20 mmol/L, pH 8.3, sodium azide; 0.95 g/L) was dispensed into each microplate well, mixed and incubated for five (5) minutes at 37^0C. Afterwards, the absorbance (A1) of was read at 340 nm using Biobase automatic biochemical microplate reader. Immediately, 50 µl of Reagent R2 (Anti-human albumin goat- polyclonal antibody, tris buffer, 50 mmol/L, pH 7.5, sodium azide 0.95 g/L) was dispensed into each of the microplate well, mixed and was read again at 340 nm after 5 minutes of incubation at 37^0C as A2 for calibrator and sample respectively.

Statistical Analysis

The statistical analysis was performed using SPSS (Statistical Package for the Social Sciences). Values was deemed significant if $p<0.01$. Correlation of the parameters with disease severity was determined using the Pearson's correlation coefficient.

Results

Table 1: Shows the sociodemographic information of the study groups via frequency table. For the test group, 14(33.3%) were within 40-49yrs of age, followed by 13(31.0%) within 50-59yrs of age, 8(19.0%) within 30-39yrs of age, 4(9.5%) within ≥60yrs of age, and 3(7.1%) within <30yrs of age; while in control, 23(54.8%) were within <30yrs of age, followed by 8(19.0%) within 30-39yrs of age, 7(16.7%) within 40-49yrs of age, 4(9.5%) within 50-59yrs of age, and 0(0.0%) within ≥60yrs of age. Both in the control and test group, 15(35.7%) and 27(64.3%) were males and females respectively. In the test group, 39(92.9%) were single, 3(7.1%) were married, and none (0, 0.0%) was neither divorced nor widow/widower; while in the control group, 23(54.8%) were married, 19(45.2%) were single, and none (0, 0.0%) was neither divorced nor widow/widower. For test group, most (26, 61.9%) have secondary education as highest educational qualification followed by 11(26.2%) having tertiary education, 4(9.5%) having primary education and 1(2.4%) having no formal education; while for the control group, 38(90.5%) have secondary education as highest educational qualification followed by 2(4.8%) having primary education, 2(4.8%) having tertiary education and none (0, 0.0%) having no formal education. Both the control and test group, 42(100.0%) each, were Igbo and Christians. With respect to occupation, 32(76.2%) were traders among the test group followed by civil servants (8, 19.0%), students (1, 2.4%), and unemployed (1, 2.4%); but in the control, 25(59.5%) were traders followed by students (16, 38.1%), civil servants (1, 2.4%), and none (0, 0.0%) was unemployed.

Table 1: Sociodemographic data of the study groups

Parameters	Categories	Test group (n = 42)	Control group (n = 42)
Age	<30yrs	3(7.1%)	23(54.8%)
	30-39yrs	8(19.0%)	8(19.0%)
	40-49yrs	14(33.3%)	7(16.7%)
	50-59yrs	13(31.0%)	4(9.5%)
	≥60yrs	4(9.5%)	0(0.0%)
Gender	Male	15(35.7%)	15(35.7%)
	Female	27(64.3%)	27(64.3%)
Marital status	Single	3(7.1%)	19(45.2%)
	Married	39(92.9%)	23(54.8%)
	Divorced	0(0.0%)	0(0.0%)
	Widow/widower	0(0.0%)	0(0.0%)

Highest educational status	Primary	4(9.5%)	2(4.8%)
	Secondary	26(61.9%)	38(90.5%)
	Tertiary	11(26.2%)	2(4.8%)
	No formal education	1(2.4%)	0(0.0%)
Ethnicity	Igbo	42(100.0%)	42(100.0%)
	Yoruba	0(0.0%)	0(0.0%)
	Hausa	0(0.0%)	0(0.0%)
	others	0(0.0%)	0(0.0%)
Religion	Christian	42(100.0%)	42(100.0%)
	Muslim	0(0.0%)	0(0.0%)
	Traditionalist	0(0.0%)	0(0.0%)
	Others	0(0.0%)	0(0.0%)
Occupation	Trader	32(76.2%)	25(59.5%)
	Civil servant	8(19.0%)	1(2.4%)
	Student	1(2.4%)	16(38.1%)
	Unemployed	1(2.4%)	0(0.0%)

Key: Statistical analysis – frequency table

Table 2: Shows the health assessment of the study groups analysed via frequency table. It showed that none (0, 0.0%) of the subjects of the control group was infected with HIV, on ART, on any other drug(s), a smoker/alcoholic, or has any underlying disease(s)/infection(s). In the test group which were only HIV patients, have 15(35.7%) being infected for 11-15yrs followed by 12(28.6%) being infected for 6-10yrs, 10(23.8%) being infected for <6yrs, 5(11.9%) being infected for ≥16yrs and none (0, 0.0%) claimed not to be infected. All the test group subjects (42, 100.0%) were on ART with 17(40.5%) being on ART for 11-15yrs, followed by 12(28.6%) being on ART for 6-10yrs, 10(23.8%) being on ART for <6yrs, 3(7.1%) being on ART for ≥16yrs and none (0, 0.0%) claimed not to be on ART. The test group subjects (42, 100.0%) claimed not to be on any other drug other than ART, not smokers/alcoholics, or having any underlying disease(s)/infection(s).

Table 3: Shows the comparison of the cystatin C levels, microalbuminuria levels, and estimated glomerular filtration rate (eGFR) between the study groups. It shows the means and standard deviations of the cystatin C levels, microalbuminuria levels, and eGFR of both test and control groups which were analysed and compared using independent samples *t*-test (significance set at $p \leq 0.05$). The cystatin C levels, and microalbuminuria levels were higher in the test group compared to that of the control group but only that of microalbuminuria levels was statistically significant

($p \leq 0.01$). However, eGFR of the control group was higher than that of the test group but was statistically non-significant ($p > 0.05$).

Table 2: Health assessment of the study groups

Parameters	Categories	Test group (n = 42)	Control group (n = 42)
Years being infected with HIV	<6yrs	10(23.8%)	0(0.0%)
	6-10yrs	12(28.6%)	0(0.0%)
	11-15yrs	15(35.7%)	0(0.0%)
	≥16yrs	5(11.9%)	0(0.0%)
	Not infected	0(0.0%)	42(100.0%)
Are you on ART?	Yes	42(100.0%)	0(0.0%)
	No	0(0.0%)	42(100.0%)
How long have you been on ART?	<6yrs	10(23.8%)	0(0.0%)
	6-10yrs	12(28.6%)	0(0.0%)
	11-15yrs	17(40.5%)	0(0.0%)
	≥16yrs	3(7.1%)	0(0.0%)
	Never	0(0.0%)	42(100.0%)
Are you on any other drug(s) other than ART?	Yes	0(0.0%)	0(0.0%)
	No	42(100.0%)	42(100.0%)
Are you a smoker/alcoholic?	Yes	0(0.0%)	0(0.0%)
	No	42(100.0%)	42(100.0%)
Do you have any of the following underlying diseases/infections?	HBV	0(0.0%)	0(0.0%)
	HCV	0(0.0%)	0(0.0%)
	Liver disease	0(0.0%)	0(0.0%)
	Tuberculosis	0(0.0%)	0(0.0%)
	CVD	0(0.0%)	0(0.0%)

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	Kidney disease	0(0.0%)	0(0.0%)
	HBP	0(0.0%)	0(0.0%)
	LBP	0(0.0%)	0(0.0%)

Key: Statistical analysis – frequency table; HBV – Hepatitis B virus; HCV – Hepatitis C virus; CVD – cardiovascular disease; HBP – High blood pressure; LBP – Low blood pressure

Table 3: Comparison of the Cystatin C levels, Microalbuminuria levels, and eGFR between the study groups

Groups	No of Subjects	Mean ± SD		
		Cystatin C (mg/L)	**Microalbuminuria (mg/dl)**	**eGFR (ml/min/1.73m^2)**
Test group	42	0.83 ± 0.33	55.52 ± 35.63	96.55 ± 30.92
Control group	42	0.80 ± 0.27	23.74 ± 14.69	97.26 ± 22.95
t-value		0.466	5.345	-0.120
p-value		0.642	0.000**	0.905

Key: Statistical analysis – independent samples *t*-test (significance set at $p \leq 0.05$); "" - Statistically significant at $p \leq 0.01$; SD – standard deviation; eGFR - estimated glomerular filtration rate**

Discussion

Previous studies have shown that infection with HIV increases the risk of different renal disorders including acute kidney injury (AKI), HIV-associated nephropathy (HIVAN), comorbid chronic kidney disease (CKD), and treatment-related kidney toxicity.[10] While the onset of HIVAN, the classic kidney disease of HIV infection, has become less common with widespread use of

antiretroviral therapy (ART); reports on the prevalence of other kidney diseases abounds. Renal dysfunction or disease is a global public health problem, affecting 750 million persons worldwide.[11] Kidney impairments in HIV-infected patients are a major cause of morbidity and mortality.[12] However, data about Cystatin C, Microalbumin and the eGFR in HIV-infected patients are still limited. Therefore, this present study assesses the renal function of HIV seropositive patients in NAUTH Nnewi using Cystatin C, Microalbumin and eGFR as biomarkers.

In this study, the serum Cystatin C level in HIV-positive individuals (test group) was not significantly different to that of HIV-negative individuals (control group), which even after further analyses based on the age groups and gender, there was still no significant difference. This is contrary to the study of Moses *et al*. [13] and John *et al*. [14] in which they reported a significant higher serum Cystatin C level in HIV-positive children and in HIV-positive adults respectively than the control group. These differences may be due to the difference in the target study population, geographical area, and sample size. Abnormal high serum Cystatin C level (> 1.15mg/L) is often used as an indication of chronic kidney disease – CKD [15] and based on the result obtained in this study, both the test and control group may be said to have normal kidney condition.

Conclusion

The inadequacy of the traditional markers in detecting early changes in GFR, and particularly in monitoring the course of advanced HIV nephropathy, calls for alternative non-invasive methods in clinical nephrology. In this study, Cystatin C seems to be an alternative and more accurate marker than Microalbumin in discriminating HIV patients with a reduced GFR as it strong correlates with the traditional eGFR.

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