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Assessment of Serum Cystatin C, Microalbumin Levels and Egfr in HIV Seropositive Individuals based on Duration 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. There were statistically non-significant differences (p> 0.05) in the cystatin C levels, and estimated glomerular filtration rate (eGFR) among the test

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group with respect to the duration of HIV infection. 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,

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

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

$N = 41.82 \approx 42$

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

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 comparison of the cystatin C levels, microalbuminuria levels, and estimated glomerular filtration rate (eGFR) among the test group with respect to the duration of HIV infection. The means and standard deviations of the cystatin C levels, microalbuminuria levels, and eGFR were analysed and compared using One-way ANOVA (significance set at $p \leq 0.05$) (Table 1a). *Post hoc* test for multiple comparisons via pairwise comparisons (significance set at $p \leq 0.05$) was used to further analysed only statistically significant variable(s) (as indicated in the ANOVA test) among three (3) or more categories or groups to identify the pair(s) of categories or groups for which the variable was statistically significant (Table 1b). There were statistically non-significant differences ($p > 0.05$) in the cystatin C levels, and estimated glomerular filtration rate (eGFR) among the test group with respect to the duration of HIV infection. While there was statistically significant difference ($p \leq 0.05$) in the microalbuminuria levels among the test group with respect to the duration of HIV infection, further analysed via *Post hoc* test which showed that those with HIV duration of <6yrs and 6-10yrs; 6-10yrs and 11-15yrs; 6-10yrs and ≥16yrs, were the pairs with significant different ($p \leq 0.05$) in their microalbuminuria levels.

Table 2: Shows the comparison of the cystatin C levels, microalbuminuria levels, and estimated glomerular filtration rate (eGFR) among the test group with respect the duration of ART. The means and standard deviations of the cystatin C levels, microalbuminuria levels, and eGFR were analysed and compared using One-way ANOVA (significance set at $p \leq 0.05$) (Table 4.10a). *Post hoc* test for multiple comparisons via pairwise comparison (significance set at $p \leq 0.05$) was used to further analysed only statistically significant variable(s) (as indicated in the ANOVA test) among three (3) or more categories or groups to identify the pair(s) of categories or groups for which the variable was statistically significant (Table 4.10b). There were statistically non-significant differences ($p > 0.05$) in the cystatin C levels, and estimated glomerular filtration rate (eGFR) among the test group with respect the duration of ART. While there was statistically significant difference ($p \leq 0.05$) in the microalbuminuria levels among the test group with respect the duration of ART, further analysed via *Post hoc* test which showed that those with ART duration of <6yrs and 6-10yrs; 6-10yrs and 11-15yrs; 6-10yrs and ≥16yrs, were the pairs with significant different ($p \leq 0.05$) in their microalbuminuria levels.

Table 1a: Comparison of the Cystatin C levels, Microalbuminuria levels, and eGFR among the test group with respect to the duration of HIV infection

Categories	No of subjects	Mean ± SD		
		Cystatin C (mg/L)	**Microalbuminuria (mg/dl)**	**eGFR (ml/min/1.73m^2)**

<6yrs	10	0.78 ± 0.47	50.60 ± 30.64	118.40 ± 44.13
6-10yrs	12	0.82 ± 0.34	81.00 ± 40.51	94.50 ± 23.20
11-15yrs	15	0.89 ± 0.28	44.53 ± 29.54	85.67 ± 22.43
≥16yrs	5	0.80 ± 0.15	37.20 ± 21.24	90.40 ± 21.97
F-value		0.264	3.601	2.653
p-value		0.851	0.022*	0.062

Key: Statistical analysis – One-way ANOVA test (significance set at $p \leq 0.05$); "*" - Statistically significant at $p \leq 0.05$; SD – standard deviation; eGFR - estimated glomerular filtration rate; HIV – Human immunodeficiency virus

Table 1b: Pairwise comparison of Microalbuminuria levels among the test group with respect to the duration of HIV infection

Paired categories	Mean difference (mg/dl)	*p*-value
<6yrs and 6-10yrs	-30.40	0.036*
<6yrs and 11-15yrs	6.07	0.652
<6yrs and ≥16yrs	13.40	0.458
6-10yrs and 11-15yrs	36.47	0.006**

6-10yrs and ≥16yrs	43.80	0.016*
11-15yrs and ≥16yrs	7.33	0.666

Key: Statistical analysis – Fisher's Least Significance Difference (LSD) *Post hoc* test (significance set at $p \leq 0.05$); "*" - Statistically significant at $p \leq 0.05$; "" - Statistically significant at $p \leq 0.01$.**

Table 2: Comparison of the Cystatin C levels, Microalbuminuria levels, and eGFR among the test group with respect to the duration of ART

Categories	No of subjects	Mean ± SD		
		Cystatin C (mg/L)	**Microalbuminuria (mg/dl)**	**eGFR (ml/min/1.73m^2)**
<6yrs	10	0.78 ± 0.47	50.60 ± 30.64	118.40 ± 44.13
6-10yrs	12	0.82 ± 0.34	81.00 ± 40.51	94.50 ± 23.20
11-15yrs	17	0.88 ± 0.27	43.76 ± 29.21	85.59 ± 22.50
≥16yrs	3	0.83 ± 0.33	36.67 ± 15.01	94.00 ± 19.70
F-value		0.214	3.572	2.699
p-value		0.886	0.023*	0.059

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Key: Statistical analysis – One-way ANOVA test along with *Post hoc* test (significance set at $p \leq 0.05$); SD – standard deviation; eGFR - estimated glomerular filtration rate; ART – Antiretroviral therapy

Discussion

Another finding in our study was that serum Cystatin C in test group tends to increase as the duration of HIV infection and ART increases but these were statistically non-significant. By contrast, Jaroszewicz *et al*. [9] reported a weak inverse relationship between serum Cystatin C and the duration of HIV infection and ART but only the duration of ART was significant. This difference may be attributed to the difference in the recruitment of the target study population based on gender, as in this study more females were recruited than males, while in the study of Jaroszewicz *et al*. [9] more males were recruited than females. Also, the difference may be due to racial difference because, Groesbeck *et al*. [10] stated that serum Cystatin C is significantly related to race/ethnicity; and this present study covers a target population of Negros while that of Jaroszewicz *et al*. [9] covers Caucasians.

In this present study, significant difference in Microalbumin level was observed with the duration of HIV infection, and the duration of ART, as Microalbumin decreases with increasing duration of HIV infection, and duration of ART. According to Montaner *et al*. [11] increase in conformity of HIV patients to effective treatment leads to decrease in HIV related morbidity as well as mortality. Therefore, the decreasing Microalbumin as duration of HIV infection, and duration of ART increases, may be due to the conformity of the test participants to their treatment which can be seen with the duration of ART similar to the duration of HIV infection; as well as the effectiveness of the ART which reduces the HIV related renal damages. However, this observation is strongly opposed by the study of Microalbumin in HIV infected children in Kano, Nigeria by Mudi *et al*. [12]where no significant difference was observed between Microalbumin and duration of HIV infection, as well as duration of ART. This contradiction may be attributed to the difference in the method of estimation as the previous study used a microalbumin specific dipstick to screen for those that are positive prior to microalbumin estimation using the albumin creatinine ratio (ACR) via immunometric (ELISA) assay and Jaffe reaction method for albumin and creatinine respectively, as compared to the direct microalbumin immunometric assay used in this present study.

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. Cystatin C and the traditional eGFR can be used to complement each other in order to give a stronger signal of kidney function and impairment in HIV patients.

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