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Assessment of Serum Cystatin C, Microalbumin Levels and Egfr in HIV Seropositive Individuals based on Age and Gender 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. More so, gender-based comparison between the test and control groups showed significantly higher levels of microalbumin in all the age groups

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(<30, 30-39yrs, 40-49yrs, 50-59yrs and ≥60yrs) of the test groups when compared to the control. Similarly, both male and female HIV-positive patients had higher microalbumin than their gender-matched 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

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.

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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 age. 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 $p \leq 0.05$). It showed that cystatin C levels was higher among those within 50-59yrs followed by those within 40-49yrs, 30-39yrs, <30yrs, and ≥60yrs in descending order. Microalbuminuria levels was higher among those within <30yrs, followed by those within ≥60yrs, 50-59yrs, 40-49yrs, and 30-39yrs in descending order. While, eGFR was higher among those within <30yrs followed by those within ≥60yrs, 30-39yrs, 40-49yrs, and 50-59yrs in descending order. Also, there was statistically non-significant difference ($p > 0.05$) in the cystatin C levels, microalbuminuria levels, and estimated glomerular filtration rate (eGFR) among the test group with respect to age.

Table 1: Comparison of the Cystatin C levels, Microalbuminuria levels, and eGFR among the test group with respect to age

Categories	No of subjects	Mean ± SD		
		Cystatin C (mg/L)	**Microalbuminuria (mg/dl)**	**eGFR (ml/min/1.73m^2)**
<30yrs	3	0.56 ± 0.36	90.67 ± 68.97	131.33 ± 38.42
30-39yrs	8	0.81 ± 0.51	48.50 ± 32.32	102.38 ± 24.63
40-49yrs	14	0.85 ± 0.29	51.43 ± 31.61	98.43 ± 38.45
50-59yrs	13	0.95 ± 0.25	54.46 ± 29.92	80.23 ± 19.32
≥60yrs	4	0.67 ± 0.16	61.00 ± 48.37	105.25 ± 14.08

F-value		1.162	0.869	2.266
p-value		0.343	0.492	0.081

Key: Statistical analysis – One-way ANOVA test (significance set at $p \leq 0.05$); SD – standard deviation; eGFR - estimated glomerular filtration rate

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

Categories	No of subjects	Mean ± SD		
		Cystatin C (mg/L)	**Microalbuminuria (mg/dl)**	**eGFR (ml/min/1.73m^2)**
Male	15	1.00 ± 0.38	48.40 ± 32.52	90.40 ± 27.39
Female	27	0.74 ± 0.27	59.48 ± 37.24	99.96 ± 32.71
t-value		2.530	-0.965	-0.959
p-value		0.015*	0.340	0.343

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

Table 3: Comparison of the Cystatin C levels between the test and control groups with respect to age and gender

Parameters	Categories	Test group (n = 42)		Control group (n = 42)		*t*-value	*p*-value
		No of subjects	**Mean ± SD (mg/L)**	**No of subjects**	**Mean ± SD (mg/L)**		
Age	<30yrs	3	0.56 ± 0.36	23	0.82 ± 0.31	-1.362	0.186
	30-39yrs	8	0.81 ± 0.51	8	0.71 ± 0.23	0.508	0.619
	40-49yrs	14	0.85 ± 0.29	7	0.85 ± 0.21	-0.035	0.973
	50-59yrs	13	0.95 ± 0.25	4	0.78 ± 0.20	1.168	0.261
	≥60yrs	4	0.67 ± 0.16	0	NA	NA	NA
Gender	Male	15	1.00 ± 0.38	15	0.84 ± 0.24	1.312	0.200
	Female	27	0.74 ± 0.27	27	0.77 ± 0.29	-0.488	0.628

Key: Statistical analysis – independent samples *t*-test (significance set at $p \leq 0.05$); SD – standard deviation; NA – Not available

Table 4: Comparison of the Microalbuminuria levels between the test and control groups with respect to age and gender

Parameters	Categories	Test group (n = 42)		Control group (n = 42)		*t*-value	*p*-value
		No of subjects	**Mean ± SD (mg/dl)**	**No of subjects**	**Mean ± SD (mg/dl)**		
Age	<30yrs	3	90.67 ± 68.97	23	29.17 ± 15.60	4.025	0.000**
	30-39yrs	8	48.50 ± 32.32	8	14.00 ± 11.36	2.849	0.013*
	40-49yrs	14	51.43 ± 31.61	7	20.14 ± 11.40	2.511	0.021*
	50-59yrs	13	54.46 ± 29.92	4	18.25 ± 7.23	2.349	0.033*
	≥60yrs	4	61.00 ± 48.37	0	NA	NA	NA
Gender	Male	15	48.40 ± 32.52	15	31.67 ± 16.53	1.777	0.086
	Female	27	59.48 ± 37.24	27	19.33 ± 11.69	5.344	0.000**

Key: Statistical analysis – independent samples *t*-test (significance set at $p \leq 0.05$); "*" - Statistically significant at $p \leq 0.05$; "" - Statistically significant at $p \leq 0.01$; SD – standard deviation; NA – Not available**

Table 5: Comparison of the eGFR between the test and control groups with respect to age and gender

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Parameters	Categories	Test group (n = 42)		Control group (n = 42)		*t*-value	*p*-value
		No of subjects	**Mean ± SD (ml/min/1.73m^2)**	**No of subjects**	**Mean ± SD (ml/min/1.73m^2)**		
Age	<30yrs	3	131.33 ± 38.42	23	101.04 ± 20.28	2.207	0.037*
	30-39yrs	8	102.38 ± 24.63	8	107.00 ± 32.72	-0.319	0.754
	40-49yrs	14	98.43 ± 38.45	7	83.86 ± 13.51	0.963	0.348
	50-59yrs	13	80.23 ± 19.32	4	79.50 ± 11.09	0.071	0.944
	≥60yrs	4	105.25 ± 14.08	0	NA	NA	NA
Gender	Male	15	90.40 ± 27.39	15	103.67 ± 16.03	-1.619	0.117
	Female	27	99.96 ± 32.71	27	93.70 ± 25.62	0.783	0.437

Key: Statistical analysis – independent samples *t*-test (significance set at $p \leq 0.05$); "*" - Statistically significant at $p \leq 0.05$; SD – standard deviation; eGFR - estimated glomerular filtration rate; NA – Not available

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Table 2: Shows the comparison of the cystatin C levels, microalbuminuria levels, and estimated glomerular filtration rate (eGFR) among the test group with respect to gender. It shows the means and standard deviations of the cystatin C levels, microalbuminuria levels, and eGFR analysed and compared using independent samples *t*-test (significance set at $p \leq 0.05$). Microalbuminuria levels and eGFR were higher in females than males but were statistically non-significant ($p > 0.05$). The cystatin C level was higher in males than in females, and was statistically significant ($p \leq 0.05$).
Table 3: Shows the comparison of the cystatin C levels between the test and control groups with respect to age and gender. The means and standard deviations were analysed and compared using independent samples *t*-test (significance set at $p \leq 0.05$). It showed that there were statistically non-significant differences ($p > 0.05$) in the cystatin C levels between the test and control groups with respect to age and gender.
Table 4: Shows the comparison of the microalbuminuria levels between the test and control groups with respect to age and gender. The means and standard deviations were analysed and compared using independent samples *t*-test (significance set at $p \leq 0.05$). Those within the age of 30-39yrs, 40-49yrs, 50-59yrs and ≥60yrs had statistically significant difference ($p \leq 0.05$) in their microalbuminuria levels with that of the test group higher than the control group; while those within the age of <30yrs had statistically significant difference ($p \leq 0.01$) in their microalbuminuria levels with that of the test group higher than the control group. In respect to gender, both the males and females of the test group had higher microalbuminuria levels than the control group but only that of females was statistically significant ($p \leq 0.01$).
Table 5: Shows the comparison of the estimated glomerular filtration rate (eGFR) between the test and control groups with respect to age and gender. The means and standard deviations were analysed and compared using independent samples *t*-test (significance set at $p \leq 0.05$). Those within the age of 30-39yrs, 40-49yrs, 50-59yrs and ≥60yrs had statistically non-significant difference ($p > 0.05$) in their eGFR between the test and control groups; while those within the age of <30yrs had statistically significant difference ($p \leq 0.05$) in their eGFR with that of the test group higher than the control group. In respect to gender, both the males and females had statistically non-significant difference ($p > 0.05$) in their eGFR between the test and control groups.

Discussion

The serum Cystatin C level with regards to age among the test group was not significantly different, but tends to increase with age. However, with respect to gender, it was found to be significantly higher in males then in females. Similarly, the study of Cystatin C level in a healthy Greek adult population by Angelos *et al*. [9] also observed that serum Cystatin C level increases with increasing age but was statistically significant; and with respect to gender was observed to be significantly higher in males then in females. Groesbeck *et al*. [10] studied the effect of age, gender and race on Cystatin C level among US adolescences, and stated that serum Cystatin C level is significantly higher in males than females, but decrease with age. The gender-based difference in this present study may be attributed to difference in growth and metabolic demands as Ziegelasch *et al*. [11] reported significant association of endocrine system, metabolites and growth factors with Cystatin C level.

Within the test group, Microalbumin was found to be non-significantly different in terms of age and gender. Microalbumin was higher in younger age than in older age as well as being higher in female than males. Similar and strongly consistent observations were made in previous studies by Molefe-Baikai *et al*. [12] and Kiconco *et al*. [13] on the prevalence of Microalbumin among diabetic patients. This may be due to nutritional and exercise status differences. High dietary intake of animal fats and proteins (which is common among younger individuals compared to older ones due to age related health issue) has been reported to increase the risk of Microalbumin.[14] Also, higher levels of physical activity/exercise associates with decrease in Microalbumin[15], hence males will tend to have lower or decreased Microalbumin than the females and vice versa.

Conclusion

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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