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Microalbuminuria and its Relationship with Clinical and Biochemical Parameters in Newly Diagnosed HIV Patients in a Tertiary Hospital South-South Nigeria

¹H.O. Okpa, ²E. Oviasu and ²L.I. Ojogwu

 ¹Department of Internal Medicine, University of Calabar Teaching Hospital, Calabar, P.M.B. 1278, Calabar, Nigeria
²Department of Internal Medicine, University of Benin Teaching Hospital, Benin, P.M.B. 1111, Benin City, Nigeria

Abstract: Microalbuminuria is a marker of vascular damage that is associated with increased risk of cardiovascular disease and mortality in the general population. Given these associations, microalbuminuria may be an important early marker of renal damage and cardiovascular risk in persons with HIV infection. Careful screening for microalbuminuria (MA) at first diagnosis of HIV may identify such patients with MA who can then be followed up.A prospective cross sectional study to determine the level of microalbuminuria and its relationship with clinical and biochemical parameters among newly diagnosed HIV patients presenting at the University of Benin Teaching Hospital, Benin City. Participants with overt proteinuria and conditions associated with microalbuminuria were excluded.Result showed that A total of 367 subjects were recruited but only 300 met the inclusion criteria with complete data for analysis and 100 aged matched HIV- negative controls. Of this number (300), 86 (28.7%) were males while 214 (71.3%) were females. Mean \pm SD ages were 37.7 \pm 9.6 and 36.1 ± 9.4 years (p = 0.142) for HIV patients and controls respectively. Microalbuminuria (urinary albumin / creatinine ratio, ACR \ge 30mg/g) was present in 44 (14.7%) of HIV subjects which is about seven times more prevalent than in controls. Several factors were associated with the development of microalbuminuria such as low CD4 count (p < 0.001), low BMI (p = 0.008), low HDL-C (p < 0.001), low GFR (p < 0.001), low Hb (p = 0.020) and high LDL-c, TC, TG (p < 0.001). But multiple linear regression analysis showed that low GFR (p = 0.010), low CD4 count (p = 0.042), low HDL-c (p = 0.004) and high LDL-c (p = 0.030) were significantly associated with the development of microabluminuria. inConclusionThis study has shown that microalbuminuria is prevalent among newly diagnosed patients with HIV infection and that there are several risk factors for yhe development of micoalbuminuria. Timely detection of this disorder and appropriate follow-up would be helpful.

Key words: Microalbuminuria • Newly Diagnosed HIV Patients • South - South

INTRODUCTION

Infection with Human immunodeficiency virus (HIV), the virus that causes Acquired immunodeficiency syndrome (AIDS) has become one of the world's most serious health and developmental challenges [1]. In sub-saharan Africa, about 22.9 million people were living with HIV in 2010 and the current prevalence in Nigeria is 3.1%.

At the end of 2010, the estimated number of people living with HIV infection was 34 million and adults accounted for more of the burden of the infection [2]. AIDS is the leading cause of death in Africa and the fourth leading cause of death worldwide [3]. At present, Nigeria has the third largest population of HIV patients worldwide after South Africa and India3. Kidney disease occurs frequently in the course of human immunodeficiency virus (HIV) infection and it has become a leading contributor to morbidity and mortality in patients with HIV and AIDS in the era of highly active antiretroviral therapy (HAART) [4]. However, it is estimated that up to 30% of HIV infected patients will have abnormal kidney function and AIDS – related kidney disease has become a relatively common cause of end-stage renal disease (ESRD) requiring dialysis and kidney disease may be associated with progression to AIDS and death [5].

Corresponding Autor: H. O. Okpa, Department of Internal Medicine, University of Calabar Teaching Hospital, P. M.B. 1278, Calabar, Nigeria.

Moreso, not much is known about the overall prevalence of microalbuminuria(MA) in HIV infected patients in the world and in Nigeria. Microalbuminuria is an indicator of endothelial or vascular injury which is associated with an increased risk of progressive renal deterioration, cardiovascular disease (CVD) and mortality [6].

There have been various studies with varying prevalence of microalbuminuria in HIV infected patients. In the pre-HAART era, microalbuminuria was reported to be prevalent in HIV infected patients with incidence ranging from 19% to 30% 6. At the moment, prevalence is reported as 8.7% to 11% [7]. A cross sectional study done in South Africa showed a prevalence of 24% for microalbuminuria in HIV patients1. HIV is an important cause of chronic kidney disease (CKD) in sub-saharan Africa with a prevalence of 38% in Nigeria [8]. A recent study done in the United states reported that, HAART has reduced the risk of end – stage renal disease (ESRD) in HIV infected patients by 40% to 60% and the 1 year survival of patients undergoing dialysis has increased from 25% to 75% [9].

Microalbuminuria is an early marker of kidney damage and is associated with increased cardiovascular and renal disease [7]. Currently, microalbuminuria is defined as the urinary albumin excretion (UAE) of 30-300mg per day, measured in a 24 hour urine collection or 20-200microgram per minute, measured in a timed urine collection, or 30-300microgram albumin/g creatinine, measured with the use of urinary albumin to creatinine ratio in a spot urine collection [10]. Currently, the exact pathogenesis of microalbuminuria is not fully understood, however there are postulations. This may be the result of altered intrarenal hemodynamics with the elaboration of wide spread low-level inflammatory processes in the body vasculature. The incidence of microalbuminuria was put at 10% in a study, but following the use of HAART there was substantial improvements in the level of microalbuminuria with about 70% having appreciable reduction and 30% having complete resolution. This therefore suggest that, microalbuminuria in HIV-infected patients may initially be the result of direct renal damage by HIV that may improve with HAART [11]. Several, but almost similar indices have been identified as predictors of microalbuminuria and overt proteinuria in HIV-infected patients but they are interwoven. The predictors of microalbuminuria include black race, older patients, higher systolic blood pressure, lower CD4 lymphocyte counts, higher HIV RNA level, higher serum creatinine level and reduced Glomerular filtration rate (GFR) less than

90mls/min [6, 12, 13]. Also,elevated levels of serum triglyceride and female gender are predictors of microalbuminuria [14]. Furthermore, microalbuminuria may be an early marker of HIVAN and as a marker of renal disease, it is associated with increased risk of cardiovascular disease and mortality in the general population and frequently seen among patients with HIV infection [13, 15]. This study was conducted to determine the prevalence of microalbuminuria in newly diagnosed HIV patients and to evaluate the relationship between microalbuminuria and clinical parameters in these patients.

MATERIALS AND METHODS

This was a single centre, observational crosssectional study of patients newly diagnosed with the HIV infection at the University of Benin Teaching Hospital, Benin city. Three hundred and sixty seven (367) newly diagnosed HIV patients were recruited for the study but only 300 patients completed the study as they met the inclusion criteria and had complete data for analysis. A hundred HIV seronegative control subjects that were aged and sex matched were recruited. Both oral and written consent was obtained from all the participants. with overt proteinuria, Patients leucocyturia, hypertensive and diabetic patients were excluded from the study. The study protocol was approved by the Research and Ethical Committee of the University of Benin Teaching Hospital (UBTH).

Clinical Evaluation and Measurements: All the participants were fully examined and anthropometric measurements such as weight, height, waist circumference,hip circumference were taken. The weight was measured with a mechanical weighing scale without shoes and with the subjects on light clothing to nearest 0.1kg. The height was measured with a stadiometre to the nearest 0.1metre (Marsdens Weighing Machine, London WA).

With the aid of a non stretch tape, the waist circumference was taken midway between the inferior margin of the last rib and the iliac crest in a horizontal plane to the nearest 0.1cm at the end of normal expiration. The hip circumference on the other hand was measured at the level of the greater trochanters with the subjects wearing light clothings to the nearest 0.1cm.

Body mass index (BMI) was then calculated as weight in kilograms divided by the square of height in metres. The ranges of BMI were categorized using the WHO classification as follows [16].

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Category	BMI range (kg/m ²)	
Severely underweight	Less than 16.5	
Underweight	16.5 to 18.4	
Normal	18.5 to 24.9	
Overweight	25.0 to29.9	
Obesity	Greater than 30	

The waist-hip ratio was also calculated by dividing waist circumference by hip circumference with normal values of ≤ 0.9 and ≤ 0.85 for males and females respectively [16].

Their blood pressures were measured in a sitting position after a 5mins rest. Blood pressure (BP) was measured on the right arm with a mercury sphygmomanometer (Accoson, England) and the systolic and diastolic blood pressures were read to the nearest 2mmHg. Disappearance of Korotkoff's sound (phase v) was the criterion for diastolic blood pressure and three consecutive readings of blood pressures were recorded at least 2 minutes apart and the mean value was used for the study. Blood pressures greater than or equal to 140mmHg systolic and 90mmHg diastolic were considered as hypertension [17].

Laboratory Evaluation: A spot urine sample of about 20mls for estimation of albumin-creatinine ratio (ACR) was collected. The concentration of albumin in urine was determined by Folin-Lowry method [18] and the urine creatinine concentration was measured by modified Jaffe's method [19]. The urine ACR was then obtained by dividing the urinary albumin concentration by the urine creatinine concentration. Spot urine samples were collected from those who have had a reasonable period of rest. Blood for serum proteins and creatinine were collected in lithium heparin bottles while that for plasma glucose was collected in fluoride oxalate bottles. Analysis for plasma glucose, creatinine and proteins were done using Hitachi 902R automated analyzer (Roche, Japan). The blood for serum lipid profile was collected in plain bottles.Serum total cholesterol (TC) and triglyceride (TG) were determined by enzymatic estimation while high density lipoprotein cholesterol l(HDL-c) was determined by enzymatic estimation after precipitation [20-22]. Low density lipoprotein cholesterol (LDL-c) was determined from the values of the aforementioned using the Friedewald's formula [23]. Blood for CD4 counts was collected in plain bottles, allowed to clot and serum collected afterwards for analysis. CD4 cell counts were analyzed by flow cytometry while the viral screens were done using the enzyme linked immunosorbent assay (ELISA) technique. The screening of subjects to determine their HIV status were made using Determine test-strips (Inverness Medical Company Ltd, USA) and HIV I/2 Stat-Pak from CHEMBIO Diagnostics, Japan.

The serum creatinine obtained was used to calculate the estimated glomerular filtration rate (eGFR) of each patient by using the Cockcroft and Gault formula24 as follows:

 $\frac{(140 - \text{Age in years}) \text{ x weight (kg) x 0.85 for women}}{72 \text{ x serum creatinine (mg/dl)}}$

This formula is simple, easy to apply and has been found to satisfactorily predict glomerular filtration rate in HIV – positive patients [25].

Data Analysis: Data was entered into and analyzed using the Statistical Package for Social Sciences (SPSS) version 17 software (Chicago, USA). Variables were summarized using frequencies and proportions for qualitative variables and mean and standard deviation for quantitative variables. The Pearson correlation was used to test for association of various variables with microalbuminuria while students t-test was used to compare continuous variables. Multiple linear regression was used to identify the independent predictors of MA. Level of significance for all significant tests was at 5% (p<0.05).

RESULTS

The characteristics of both patients and controls are shown in Table 1. The mean age in years of the participants were 37.7 (\pm 9.6) in HIV subjects and this was not statistically different from that of the controls which was 36.1 (\pm 9.4) years (P = 0.142). The mean BMI was significantly higher in HIV subjects 23.7 (± 4.6)kg/m2 when compared with controls $22.9 (\pm 2.2)$ kg/m2 (P=0.018). The mean WHR is 0.89 (±0.03) in HIV subjects and 0.90 (± 0.02) in controls with no significant difference (P = 0.104). Also, there were no significant differences in the BMI and WHR among males and females in HIV subjects and controls. The mean diastolic blood pressures was 75 (±8.2)mmHg in HIV subjects and was significantly lower than the value of 77.9 (\pm 7.6) mmHg in controls (P = 0.001). The mean urine albumin-creatinine ratio (ACR) was 14.6 (±21.7)mg/g in HIV subjects and was significantly higher than 6.7 (± 5.6) mg/g for controls (P < 0.001). The mean total serum proteins and albumin in HIV subjects were 6.8 $(\pm 0.4)g/l$ and 3.6 $(\pm 0.3)g/l$ respectively and these values were significantly lower

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Table 1: Clinical and laboratory characteristics of HIV subjects and controls

Variable	HIV subjects (n=300)	Controls (n=100)	p-value
Age(years)	37.7±9.6	36.1±9.4	0.102
Gender			
Male	86(28.7)	38(38.0)	0.106
Female	214(71.3)	62(62.0)	
Body mass index (BMI)kg/m2	23.7±4.6	22.9±2.2	0.018
Male	23.9±3.6	23.0±2.0	0.149
Female	23.7±4.9	22.9±2.3	0.225
Waist hip ratio (WHR)	0.89±0.03	0.90±0.02	0.104
Male	0.90±0.03	0.91±0.01	0.228
Female	0.89±0.03	0.89 ± 0.02	0.799
Systolic blood pressure (mmHg)	113.8±8.0	114.9±8.6	0.247
Diastolic blood pressure (mmHg)	75±8.2	77.9±7.6	0.001
Urine albumin- creatinine ratio (mg/g)[ACR]	14.6±21.7	6.7±5.6	< 0.001
Total serum proteins (g/l)	6.8±0.4	7.6±0.5	< 0.001
Serum albumin (g/l)	3.6±0.3	4.3±0.4	< 0.001
Packed cell volume (%)[PCV]	33.5±4.7	38.2±3.4	< 0.001
Serum creatinine (µmol/l)	80.4±15.0	76.9±11.5	0.005
Glomerular filtration rate (mls/min)[GFR]	89.2±16.2	95.3±10.0	< 0.001
Lipid profile (mmol/l) Total cholesterol	3.8±0.5	4.1±0.4	< 0.001
HDL-cholesterol	0.9±0.3	1.0±0.2	< 0.001
LDL-cholesterol	2.2±0.4	2.6±0.4	< 0.001
Triglyceride	1.6±0.8	1.1±0.2	< 0.001

ACR=Albumin-creatinine ratio, HDL=High density lipoprotein,LDL=Low density lipoprotein

Table 2: Clinical and biochemical characteristics of HIV subjects with MA and HIV subjects without MA.

Variable	HIV subjects with MA (n=44)	HIV subjects without MA (n=256)	p-value
Age (years)	39.8±11.47	37.3±9.25	0.186
Gender			
Male	86(28.7)	38(38.0)	0.106
Female	214(71.3)	62(62.0)	
Body mass index (BMI)kg/m2	21.8±3.55	24.1±4.69	< 0.001
Male	22. 8±2.50	24.1±3.81	0.103
Female	21.3±3.96	24.05±4.98	0.002
Waist hip ratio (WHR)	0.88±0.027	0.89±0.028	0.296
Male	0.904±0.023	0.903±0.029	0.837
Female	0.88±0.025	0.89±0.027	0.059
Systolic blood pressure (mmHg)	113.4±6.45	113.8±8.22	0.704
Diastolic blood pressure (mmHg)	74.5±7.3	75.0±8.31	0.686
Total serum proteins (g/l)	6.77±0.46	6.80±0.36	0.713
Serum albumin (g/l)	3.5±0.28	3.62±0.30	0.014
Packed cell volume (%)[PCV]	33.4±4.92	33.7±4.74	0.097
Serum creatinine (µmol/l)	83.3±14.2	80.6±15.1	0.212
Haemoglobin (mg/dl)	10.58±1.90	11.14±1.78	0.074
Glomerular filtration rate (mls/min)[GFR]	78.7±13.2	91.0±16.0	< 0.001
Lipid profile (mmol/l) Total cholesterol	4.33±0.53	3.76±0.39	< 0.001
HDL-cholesterol	0.69±0.14	0.93±0.27	< 0.001
LDL-cholesterol	2.67±0.46	2.11±0.34	< 0.001
Triglyceride	2.08±0.52	1.1-57±0.78	< 0.001

HDL=High density lipoprotein,LDL=Low density lipoprotein.

than the values of 7.6 (±0.5)g/l (P < 0.001) and 4.3 (±0.4g)/l (P < 0.001) in controls. The mean PCV in HIV subjects was 33.5 (±4.7)% and was significantly lower than 38.2 (±3.4)% in controls, (P < 0.001). Also, mean serum creatinine and GFR were 80.4 (±15.0)µmol/l and 89.2 (±16.2)mls/min respectively in HIV subjects in which the creatinine value was significantly higher than 76.9 (±11.5)µmol/l (P = 0.005) while GFR value was significantly lower than 95.3

(±10)mls/min in controls (P < 0.001). Moreso, the mean total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly lower in HIV subjects as compared to controls (P < 0.001) while the mean TG was significantly higher in HIV subjects (P < 0.001).

The clinical and biochemical parameters of HIV patients with MA and those without MA are shown in Table 2. The mean age of HIV patients with MA is not

Variable	Correlation (R value)	p-value
Age (years)	0.056	0.334
BMI (kg/m ²)	-0.153**	0.008
WHR	-0.069	0.232
Systolic blood pressure (mmHg)	-0.019	0.748
Diastolic blood pressure (mmHg)	-0.021	0.711
CD4cell count	-0.206**	< 0.001
Creatinine (µmol/l)	0.068	0.242
GFR (mls/min)	-0.263**	< 0.001
Serum albumin (g/l)	-0.120**	0.038
Haemoglobin (mg/dl)	-0.135*	0.02
Total cholesterol (mmol/l)	0.399**	< 0.001
HDL-cholesterol (mmol/l)	-0.302**	< 0.001
LDL-cholesterol (mmol/l)	0.451**	< 0.001
Triglyceride (mmol/l)	0.228**	< 0.001

Table 3: Correlation of microalbuminuria with clinical and laboratory

parameters in HIV patients

Hb= Haemoglobin, HDL=High density lipoprotein, LDL=Low density lipoprotein, BMI=Body mass index, GFR=Glomerular filtration rate. Bolded values are significant.**=correlation is significant at the 0.01 level.*=correlation is significant at the 0.05 level.

Table 4: Multiple linear regression analysis showing predictors of Microalbuminuria in HIV patients

Variable	Beta	t	p-value
BMI (kg/m ²)	-0.08	-1.223	0.222
CD4 count (cells/µl)	-0.103	-2.043	0.042
eGFR (mls/min)	-0.135	-2.598	0.01
Albumin (g/l)	0.015	0.23	0.818
Hb (mg/dl)	0.057	0.401	0.689
TC (mmol/l)	0.268	1.651	0.1
HDL-c (mmol/l)	-0.305	-2.909	0.004
LDL-c (mmol/l)	0.312	2.18	0.03
TG (mmol/l)	0.071	0.535	0.593

Model Summary : R= 0.655, R²= 0.429, P= <0.000

statistically different from those without MA (39.8±11.47 vs 37.3 \pm 9.25, P = 0.186). The mean BMI is significantly lower in HIV patients with MA as compared to those without MA (21.8 ± 3.55 vs 24.1 ± 4.69 , P < 0.001). Also, the mean CD₄ count, eGFR and serum albumin levels were significantly lower in HIV patients with MA as compared to those without MA. On the other hand, the lipid parameters are such that the total cholesterol, LDL cholesterol, TG levels were significantly higher in HIV patients with MA as compared to those without MA (P <0.001). However, HDL – cholesterol was significantly lower in HIV patients with MA than those without MA $(0.69\pm0.14 \text{ vs } 0.93\pm0.27, P < 0.001)$. Moreso, the WHR, systolic and diastolic blood pressures, the creatinine, proteins, packed cell volume and haemoglobin levels were not significantly different in the two groups (P > 0.05).

In Table 3, with microalbuminuria as the dependent variable, the levels of BMI, CD4 count, GFR, serum albumin, Hb and HDL-cholesterol negatively correlated with MA (P < 0.05) while LDL-cholesterol, total cholesterol, triglyceride, correlated positively with MA (P < 0.01). There was no correlation between age, WHR and creatinine levels with MA (P > 0.05).

Multiple linear regression analysis showing the independent predictors of MA, with MA as the dependent variable and other parameters as independent variables. The independent variables regressed were those found to have correlated significantly with MA. Low CD4 count, GFR, HDL-cholesterol and high LDL-cholesterol levels were predictors of MA in the model. The results are as shown in Table 4.

DISCUSSION

Microalbuminuria, an early marker of renal disease was present in about 14.7% of all subjects with HIV infection. The mean CD4 cell count in all the HIV subjects in our study was low and this agrees with other studies [12, 13]. Also, the mean CD4 cell count was significantly lower in HIV subjects with MA compared to those without MA. This finding is in keeping with reports elsewhere but different from the report from a study that did not find any association between CD4 count levels and MA [6, 12, 13]. In that study, majority of the patients had a longer duration of illness and have been on HAART for a period of greater than a year while in our study majority of the patients studied were asymptomatic individuals.

In this study, serum creatinine levels was high in HIV subjects and this was even higher in HIV subjects with MA as compared to those without MA but there was no significant association. The difference observed may likely be due to the category of patients selected, as most of the patients in the other study were symptomatic with features of AIDS.

The GFR is significantly lower in HIV patients with MA as compared to those without MA. This is similar to findings in reports where GFR<90mls/min was a predictor of the development of MA [12, 13].

The association of GFR with MA was corroborated in this study as GFR was negatively correlated with MA. Also, GFR independently predicted MA in this study which is in keeping with other studies done elsewhere but in contrast to a study where more than 60% of the patients have been on ARTs for at least a year [6, 12, 13].

The mean serum albumin is significantly low in the HIV patients. This finding is similar to reports in available literatures [28, 29]. In these studies, low serum albumin level was found to be important predictor of morbidity in patients with AIDS [28, 29]. The low serum albumin in HIV patients may be due to the malnutrition associated with AIDS and HIV infection which may present in the severe form with HIV wasting syndrome [30-32]. Feldman et al. [29] also showed that serum abumin correlated positively with low CD4 cell count and in our study, CD4 cell count was signicantly correlated with MA. Also, low serum albumin was significantly associated with the development of MA.

The lipid profile abnormalities were variable in keeping with reports from other studies [33, 34]. The HIV subjects in this study had low levels of TC, HDL-c, LDL-c and elevated levels of TG. This pattern is similar to reports obtained elsewhere [33, 34]. However, among HIV subjects with MA as compared to those without MA the pattern of lipid disorders were different in which the patients with MA had elevated TC, LDL-c, TG and low HDL-c.

In this study, high levels of TC, LDL-c and TG were significantly correlated with MA while low levels of HDL-c was significantly correlated with the outcome of MA. This is in keeping with reports from Pantelis *et al.* [10] and Szczech *et al.* [14]. Moreso, low levels of HDL-c and high levels LDL-c were independent predictors of MA in this study but this was not so in other studies where majority of the patients were already on ARTs [10, 13].

This study revealed that majority of the HIV subjects had normal BMI but the values of BMI were however lower in HIV patients with MA. The significant association of low BMI with MA is in contrast with the findings from other reports [10, 11]. Perhaps the chronic nature of HIV infection with associated malnutrition may explain this finding.

There were no significant association between age, WHR, blood pressure and MA. A study by Szczech *et al.* [14] revealed that older age is a predictor of MA in HIV patients [13]. However, HIV patients in our study with MA were older than their counter parts without MA but this was not significant. The mean SBP and DBP were low in keeping with some reports [8, 35]. The mean haemoglobin and PCV were lower in the HIV subjects in keeping with findings from a report [36]. The lower haemoglobin and PCV values probably reflect the chronic nature of HIV infection associated with chronic malnutrition. In this study low Hb was associated with MA.

CONCLUSION

This study has shown that low CD4 count levels, low serum albumin levels, low BMI, low Hb, lipid profile disorders and low GFR levels were the most significant risks factors for the development of microalbuminuria in this study. However, the independent predictors of microalbuminuria were low CD4 counts, low GFR levels, low HDL-C and elevated LDL-C levels.

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