Short communication

Does HAART improve renal function? An association between serum cystatin C concentration, HIV viral load and HAART duration

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Aim: The prevalence of renal disease in human HIV-infected individuals varies between 2% and 10%. Many reports have demonstrated the beneficial effect of antiretroviral (ARV) therapy on slowing the progression of renal diseases. The aim of our cross-sectional study was to determine serum cystatin C concentration in different stages of HIV infection and the relationship between cystatin C concentration and ARV treatment.

Methods: Cystatin C concentration was measured in the sera of 77 HIV-1-infected individuals and 18 HIV-seronegative volunteers. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease Study formula.

Results: HIV infection resulted in a significant increase in serum cystatin C concentration compared with healthy individuals (933.4 ±32.1 vs 621.1 ±56.8 ng/ml, $P<0.001$). There were no significant differences in urea, creatinine and GFR between those groups. On multivariate analyses serum cystatin C was independently associated with highly active antiretroviral therapy (HAART) duration ($\beta=-0.34$, $P=0.04$) and HIV viral load ($\beta=0.33$, $P=0.04$), whereas there were no significant relationships with age, body mass index, HIV duration, $CD4^+$ and $CD8^+$ T-cell counts and serum high sensitivity C-reactive protein concentration.

Conclusions: Our initial observations indicate that serum cystatin C, which may reflect mild renal dysfunction, is increased during HIV-infection and is associated with HIV viral load. Long-lasting HAART seems to decrease cystatin C concentration, thus potentially improves renal function.

Introduction

The prevalence of renal disease in HIV infection varies between 2% and 10% [1,2]. The most common renal disorder in HIV is HIV-associated nephropathy, caused by direct infection of renal epithelium by HIV [3]. Other renal diseases include immunoglobulin A nephropathy, cryoglobulinaemia, amyloidosis and a lupus-like immune complex glomerulopathy [4].

Many reports demonstrated beneficial effect of antiretroviral (ARV) therapy on slowing progression of renal diseases [5,6]. Szczech *et al.* [7] showed favourable effect of ARV therapy including protease inhibitors on progression HIV-associated nephropathy. Moreover, a recently published 12-year cohort study demonstrated substantial reduction in HIV-associated nephropathy incidence associated with highly active antiretroviral therapy (HAART) [8]. By contrast, it is noteworthy that some ARV drugs, especially tenofovir, yield nephrotoxic effect and may cause renal dysfunction [9].

Cystatin C is a non-glycosylated low molecular weight (13 kDa) basic protein that is a member of the cystatin superfamily of cysteine protease inhibitors. It is a secreted protein ubiquitously expressed in all tissues. Therefore, it has a stable production rate even if there is an inflammatory response, and is freely filtered by the glomeruli. Moreover, as opposed to serum creatinine, serum cystatin C concentration is not affected by dietary protein intake, and there is no interference of proteins and bilirubin present in serum during the estimation process. Several investigators have previously reported that cystatin C concentration in serum correlates well with glomerular filtration rate (GFR) [10–12].

The aim of our study was to investigate the possible relationship between antiretroviral treatment and renal function expressed by cystatin C serum concentration.

Patients and methods

A cross-sectional, initial exploratory study was performed in 77 HIV-1-infected individuals (55 male, 22 female, aged 20–53 years, mean 34.1). Mean HIV infection duration was 6.2 years (minimum 6 months, maximum 16 years). Fifty-nine individuals were given HAART including at least one protease inhibitor and nucleoside reverse transcriptase inhibitors. The mean HAART duration was 36.2 months (minimum 1 month, maximum 95 months). None of the study participants received tenofovir. Individuals were categorized according to 1993 Centres for Disease Control and Prevention (CDC) classification [13], with 7 individuals in stage A, 43 in stage B and 27 in stage C. Individuals with known renal disorders, proteinuria, diabetes mellitus, hypertension or a recent history of nephrotoxic drug administration were not included in the study.

The control group consisted of 18 healthy, HIV-negative volunteers with ages ranging from 27 to 45 years (mean 35.8 years). Ethical approval for the study was obtained from the Bioethical Committee of the Medical University of Bialystok. Informed consent was obtained from every individual included in the study. All individuals who were included were Caucasian race.

The percentage and absolute counts of peripheral $CD4^+$ and $CD8^+$ T cells were determined by means of three-colour flow cytometric analysis (Beckton–Dickinson, Franklin Lakes, NJ, USA). Plasma HIV-1 RNA was evaluated using the Amplicor system (Roche Diagnostics, Basel, Switzerland), with sensitivity ranging from 50 to 100,000 RNA copies per ml.

Cystatin C serum concentration was measured by sandwich enzyme immunoassay (BioVendor GmbH, Heidelberg, Germany). According to manufacturer information, the cystatin C assay limit of detection is 0.2 ng/ml, intra-assay coefficient of variation (CV) is 5.0–9.6% and inter-assay CV is 4.8–6.2%. Creatinine, urea and albumin concentrations were measured in serum. Proteinuria was quantified by dipstick tests (MULTISTIX 10SG, Bayer Diagnostics Division, Tarrytown, New York, USA). The GFR was estimated using the Modification of Diet in Renal Disease Study formula: GFR=170×[serum creatinine concentration (mg/dl)]$^{-0.999}$×[Age]$^{-0.176}$×[0.762 if individual is female]×[serum urea nitrogen (mg/dl)]$^{-0.17}$×[serum albumin concentration (g/dl)]$^{+0.318}$ with normal range of 90–120 ml/min per 1.73m^2 [14]. Serum C-reactive protein (CRP) concentration was measured by a high-sensitivity enzyme-linked immunosorbent assay (Imuclone, Stamford, CT, USA) with dilution 1:100, according to manufacturer instructions.

Statistical analyses

Measured values were expressed as means and standard errors of the mean (±SE). Significance of differences between studied groups was calculated by non-parametric Mann–Whitney *U* test. For correlation analyses, the Spearmann non-parametric correlation was used. Multivariate analyses were carried out by a step-wise logistic regression model. A *P*-value <0.05 was considered significant. Statistical analyses were performed with Statistica 5.0 for Windows software (Statsoft Inc., Tulsa, USA).

Results

Cystatin C concentration was measured in serum of 77 HIV-1-infected individuals and 18 HIV-seronegative volunteers. The clinical characteristics of studied group are shown in Table 1. HIV-infection resulted in a significant increase in serum cystatin C concentration compared with healthy individuals (933.4 ±32.1 vs 621.1 ±56.8 ng/ml, $P<0.001$). There were no significant differences in urea, creatinine and GFR estimation between those groups (Table 1). Moreover, there were no associations between $CD4^+$, $CD8^+$ as well as nadir $CD4^+$ T-cell count and serum cystatin C. By contrast, we observed a significant, positive correlation of cystatin C with HIV-1 viral load ($r=0.39$, $P<0.001$).

The highest serum cystatin C values were observed in individuals without ARV treatment, whereas the lowest concentrations were detected in the HAART group; however, those differences were not significant (Table 1). There were no differences in cystatin C HAART patients when adjusted to ARV regiment. Interestingly, cystatin C levels were influenced by HAART duration, expressed by a significant, negative correlation ($r=-0.34$, $P=0.01$).

Despite the lack of correlation with $CD4^+$ T-cell count, serum cystatin C concentration was increased in late-stage symptomatic patients (CDC C: 1,041.4 ±64.4 ng/ml) compared with those in CDC B (857.4 ±36.3 ng/ml, $P=0.01$) and CDC A (934.9 ±69.3 ng/ml, $P=0.48$).

Serum CRP concentration, measured by a high sensitivity enzyme-linked immunosorbent assay was almost fourfold increased in HIV-infected individuals compared with the control group (16.5 ±2.3 vs 4.4 ±1.4 µg/ml, $P=0.05$). There were no significant differences in CRP levels between individuals receiving HAART and those without antiretroviral treatment (Table 1).

On multivariate analyses serum cystatin C was independently associated only with HAART duration

(β=-0.34, *P*=0.04) and HIV viral load (β=0.33, *P*=0.04), whereas there were no significant relationships with age, BMI, HIV-duration, $CD4^+$, $CD8^+$ counts and serum CRP concentration (Table 2). There was no significant association between GFR and serum cystatin C (β=0.06, *P*=0.71).

Discussion

The usefulness of cystatin C monitoring as a marker of GFR has been validated in many disorders, including in individuals undergoing cardiac catheterization, in diabetes and in critically ill patients [15–17]. Of particular value, it is more sensitive than serum creatinine in the detection of early renal insufficiency in a range of renal disorders [18,19]. Nevertheless, some recent reports showed that cystatin C measurement was limited as a marker of GFR, especially following organ transplantations, in leukaemia and during pregnancy [20,21]. There are only scarce data concerning its applicability in HIV infection.

HIV-infected individuals included in our study did not present significant differences of serum creatinine, urea and GFR estimation compared with healthy individuals. There was no significant association between GFR and serum cystatin C, which may be a result of GFR within normal range observed in

Table 1. Clinical characteristics as well as serum concentrations of cystatin C, creatinine, urea and GFR in the studied population

	Control group *(n=18)*	HIV+ *(n=77)*	*P*-value	HAART– *(n=18)*	HAART+ *(n=59)*	*P*-value
Age, years	35.8 ±1.3	34.1±0.88	0.34	32.2 ±1.9	34.7 ±0.9	0.29
Sex, male/female	10 / 8	55 / 22	0.19	13 / 5	42 / 17	0.93
BMI	22.4 ±0.5	23.3 ±0.4	0.33	24.6 ±1.9	23.1 ±0.5	0.24
HIV infection duration, years	–	6.22 ±0.5	–	4.8 ±0.9	6.6 ±0.5	0.07
$CD4^+$ T-cell count, cells/ml	–	400.3 ±30.6	–	389.7 ±95.9	403.6 ±27.9	0.14
Nadir $CD4^+$ T-cell count, cells/ml	–	152.8 ±14.8	–	299.1 ±36.9	107.6 ±9.6	<0.001
$CD8^+$ T-cell count, cells/ml	–	1,007.3 ±56.4	–	1,011.7 ±130.1	1,006.5 ±62.9	0.90
HIV viral load, copies/ml	–	19,672.1 ±4,011.8	–	31,019 ±8,342	16,287 ±4,518	<0.001
Cystatin C, ng/ml	621.1 ±56.8	933.4 ±32.1	<0.001	992.4 ±63.2	915.5 ±37.1	0.15
Creatinine, mg/dl	0.69 ±0.03	0.75 ±0.02	0.20	0.71 ±0.05	0.77 ±0.03	0.19
Urea, mg/dl	28.6 ±2.7	30.1 ±2.1	0.78	25.6 ±2.6	30.9 ±2.4	0.45
GFR estimation*, ml/min/1.73m^2	115.2 ±8.9	103.8 ±8.7	0.06	137.5 ±29.9	93.4 ±6.3	0.26
CRP high sensitivity, μg/ml	4.4 ±1.4	16.5 ±2.3	0.05	14.7 ±4.7	17.0 ±2.7	0.25

*Calculated by Modification of Diet in Renal Disease Study formula: GFR=170 × [serum ceratinine concentration (mg/dl)]$^{-0.999}$×[Age]$^{-0.176}$×[0.762 if individual is female]×[serum urea nitrogen (mg/dl)]$^{-0.17}$×[serum albumin concentration (g/dl)]$^{+0.318}$. Data are presented as mean ±standard error of mean. BMI, body mass index; CRP, C-reactive protein; GFR, glomerular filtration rate; HAART, highly active antiretroviral treatment;

Table 2. The relationship between serum cytostatin C, glomerular filtration rate and creatinine in the studied population

	Cystatin C (ng/ml)		GFR estimation* (ml/min per 1.73m^2)		Creatinine (mg/dl)	
	β	*P*-value	β	*P*-value	β	*P*-value
Age, years	-0.33	0.06	-0.11	0.54	-0.07	0.70
BMI	-0.13	0.46	0.26	0.12	-0.24	0.14
HIV duration, years	-0.07	0.68	0.02	0.99	0.27	0.11
HAART duration†, months	-0.32	0.04‡	0.03	0.86	-0.18	0.31
Nadir $CD4^+$ T-cell count, cells/ml	-0.21	0.22	0.21	0.21	0.77	0.66
CD4 count$^+$, cells/ml	0.06	0.97	0.12	0.47	0.79	0.65
CD8 count$^+$, cells/ml	0.01	0.99	-0.10	0.55	-0.03	0.87
HIV viral load, copies/ml	0.33	0.04‡	0.04	0.79	-0.05	0.75
CRP (high sensitivity), μg/ml	-0.01	0.96	-0.18	0.31	0.01	0.99

The relationship between serum cystatin C, glomerular filtration rate (GFR) and creatinine (independent variables) as well as age, body mass index, nadir $CD4^+$, $CD4^+$, $CD8^+$ T-cell counts, HIV viral load and high sensitivity C-reactive protein concentration (dependent variables) in the studied population, as estimated by a stepwise multivariate regression model. *Calculated by Modification of Diet in Renal Disease Study formula: GFR=170×[serum ceratinine concentration (mg/dl)]$^{-0.999}$×[Age]$^{-0.176}$×[0.762 if individual is female]×[serum urea nitrogen (mg/dl)]$^{-0.17}$×[serum albumin concentration (g/dL)]$^{+0.318}$. †Includes individuals with ongoing highly active antiretroviral therapy (HAART; *n*=59). ‡Denotes statistical significance, *P*<0.05. BMI, body mass index

studied population. On the contrary, we observed significantly increased serum cystatin C in HIV, which may indicate previously unrecognized renal dysfunction in those patients.

The kidney is one of the HIV reservoirs, where virus can exert both direct and indirect pathogenic activity. We showed a positive correlation between serum cystatin C and HIV-1 viral load. It was shown recently that the HIV viral protein Nef induces the early molecular changes in podocytes. These changes are essential for the differentiation and proliferation of podocytes in HIV-associated nephropathy pathogenesis [22]. Winston *et al.* [23] described reversal of histological changes in HIV-associated nephropathy after obtaining HIV viral suppression. Additionally, Kimmel *et al.* [24] also suggested a relationship between HIV viral load and renal dysfunction. By contrast, Izzedine *et al.* [25] described a case of HIV-associated nephropathy in an individual with undetectable HIV RNA, which suggests that this disorder may occur at any stage of disease.

Another finding in our study was decreased serum cystatin C in patients undergoing HAART; however, the difference was not significant. Furthermore cystatin C levels correlated inversely with ARV duration. This may indicate favourable effect of longitudinal ARV therapy on renal function in HIV infection.

We observed significant increased serum cystatin C in late-stage HIV disease (CDC C group). Patients included in our study did not present symptoms of ongoing AIDS-defining conditions and CDC C stage was diagnosed on the basis of previous disorders. A possible reason for renal dysfunction in this group could be nephrotoxic drug regiment in the past. Many authors have suggested nadir $CD4^+$ T-cell count as a risk factor of development and progression of renal disorders [26]. The lack of association between this parameter and serum cystatin C may be explained by discordance between immunological and clinical stage of HIV infection. This observation indicates the need of renal function monitoring particularly in late-stage disease.

Regardless of the promising results of our study showing a potentially favourable effect of longitudinal ARV therapy on kidney function as well as a negative association between serum cystatin C and HIV viral load, some limitations have to be addressed. Taking into consideration the cross-sectional nature of our study and fact that cystatin C is secreted by various tissues, is it extremely difficult to draw firm conclusions. The recent work of Rule *et al.* [27] suggested that cystatin C may be affected by ongoing inflammation. In our study, multivariate analysis did not show an association between hsCRP (high sensitivity CRP) values and cystatin C. However, the results of this study need confirmation in controlled, longitudinal settings, and we propose to consider our study as initial and exploratory.

In conclusion, our initial observations indicate that serum cystatin C, which may reflect mild renal dysfunction, is increased during HIV-infection and associated with HIV viral load. Long-lasting HAART seems to decrease cystatin C concentration, thus potentially improving renal function.

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