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Proteomics analysis of blood plasma in HIV-infected patients with chronic kidney disease

Amanda Anne Lavinya[a], Cheng Siang Lee[b], Onn Haji Hashim[c,d], Iskandar Azwa[e], Reena Rajasuriar[a,b], Soo Kun Lim[f], Yuen Fei Wong[a,*]

[a] Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Centre of Excellence for Research in AIDS, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] University of Malaya Centre for Proteomics Research, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[d] Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[e] Infectious Diseases Unit, Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[f] Nephrology Unit, Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

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ABSTRACT

Background: Patients treated for human immunodeficiency virus (HIV) infection are prone to developing chronic kidney disease (CKD). Current methods used in assessing kidney function suffer inaccuracy in HIV-infected patients. This study aims to identify biomarkers that could complement existing methods of kidney assessment among HIV-infected subjects.

Methods: Plasma protein profiling was performed for HIV patients with CKD presented with negative/trace proteinuria (non-proteinuric) (n = 8) and their matched non-CKD controls, using two-dimensional gel electrophoresis (2DE); selected protein candidates were identified using mass spectrometry. Subsequently, altered plasma abundance of protein candidates were verified using Western blotting in HIV-infected subjects with non-proteinuric CKD (n = 8), proteinuric CKD (n = 5), and their matched non-CKD controls, as well as in HIV-uninfected subjects with impaired kidney function (n = 3) and their matched controls.

Results: Analysis of 2DE found significantly altered abundance of five protein candidates between HIV-infected patients with non-proteinuric CKD and without CKD: alpha-1-microglobulin (A1M), serum albumin (ALB), zinc-alpha-2-glycoprotein (AZGP1), haptoglobin (HP), and retinol binding protein (RBP4). Western blotting showed an increased abundance of A1M and HP in HIV-infected patients with non-proteinuric CKD compared to their non-CKD controls, whereas A1M, AZGP1, and RBP4 were significantly increased in HIV-infected patients with proteinuric CKD compared to their non-CKD controls. Such pattern was not found in HIV-uninfected subjects with impaired kidney function.

Conclusion: The data suggests four proteins that may be used as biomarkers of CKD in HIV-infected patients. Further validation in a larger cohort of HIV-infected patients is necessary for assessing the clinical use of these proposed biomarkers for CKD.

1. Introduction

Individuals infected with human immunodeficiency virus (HIV) suffer an increased risk of developing noncommunicable diseases, despite the near normal life expectancy that can be achieved with antiretroviral therapy [1]. Many people living with HIV (PLHIV) develop chronic kidney disease (CKD), defined as the 'abnormalities of kidney structure or function, present for > 3 months, with implications for health' [2]. The first kidney pathology reported in HIV patients was HIV-associated nephropathy (HIVAN). Patients with HIVAN often present with collapsing focal segmental glomerulosclerosis, tubular microcyst formation, tubulointerstitial inflammation, and kidney fibrosis, which rapidly progresses to end stage renal disease (ESRD) [3]. Since the widespread use of ART to treat HIV, HIVAN has become less common [3]. However, CKD has fast become an important cause of morbidity and mortality among PLHIV as non-HIVAN kidney diseases such as HIV-immune complex kidney disease, diabetic nephropathy, and drug-induced nephropathy become more prevalent with longer survival [3].

For many years, the guidelines for diagnosis, prevention and

* Corresponding author at: Department of Pharmacy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail address: yfwong@um.edu.my (Y.F. Wong).

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management of CKD among PLHIV were largely adopted from those used for the general populations [4]. In March 2017, a multidisciplinary international panel of clinical and scientific experts, convened by the Kidney Disease: Improving Global Outcomes (KDIGO) organization, had outlined numerous issues and knowledge gaps in the existing practice, including the accuracy and specificity of standard serum creatinine-based estimated glomerular filtration rate (eGFR) and serum cystatin C-based examination in assessing kidney function in the HIV population [3]. The use of such markers as surrogates for kidney function among PLHIV may not be ideal, due to altered metabolism, body mass abnormalities, and exposure to multiple medications known to affect renal tubular creatinine and cystatin C secretion in this disease population [5]. While the presence of urinary protein also indicates kidney functional or structural defects, non-proteinuric CKD presents an even higher risk of disease progression [6], as the disease pathology is poorly understood hence no effective treatment is available to date. In fact, in HIV-infected subjects, proteinuria, when used as an independent marker, is not a strong predictor for risk stratification of ESRD [7]. Due to the limitations of using eGFR and proteinuria tests in estimating kidney function among PLHIV, researchers have been studying alternative biomarkers in this specialized population, including plasma FGF-23 [8], urinary KIM-1 [9], and urinary NGAL [9], that remain to be validated in a wider population of HIV-infected individuals.

Proteomics study, specifically gel electrophoresis, has been employed to discover biomarkers associated with HIV infection itself, as well as those associated comorbidities, such as cognitive dysfunction [10]. However, the difference in the plasma proteome of HIV-infected individuals with and without CKD remains largely unexplored. In fact, there is only one known urinary proteomics study in childhood HIV-associated renal diseases to date [11]. In this study, we aimed to identify proteins of altered abundance between HIV-infected patients on stable ART with and without CKD that may be used as biomarkers for kidney dysfunction in the HIV-infected population.

2. Materials and methods

2.1. Subjects and samples

Plasma samples used in this study were previously collected and stored upon recruitment of the Malaysian HIV and Aging (MHIVA) cohort at University Malaya Medical Center (UMMC) (Supporting Information 1) [12]. All clinical and demographic information were obtained from the UMMC patient medical records. Subjects for our study were selected based on their eGFR values, namely those with two or more eGFR values of < 60 mL/min/1.73 m^2 were categorized as CKD cases while those with two or more eGFR values of 90–125 mL/min/1.73 m^2 were categorized as non-CKD cases. Among the HIV-infected subjects enrolled in the MHIVA study, 13 were identified to have CKD. Of these CKD subjects, one had HIVAN [3], diagnosed nine months prior to sample collection; another had Fanconi syndrome, diagnosed 3.5 years prior to sample collection, that describes a generalized disorder in the proximal renal tubule transport leading to renal wasting of a wide range of molecules, including amino acids, glucose, phosphate, and uric acid [13]. Another 13 HIV-infected individuals without CKD, who were matched for age, gender, and comorbidities (specifically hypertension and diabetes mellitus) to the CKD subjects, were selected. HIV-uninfected individuals with low (< 60 mL/min/1.73 m^2) ($n = 3$) and normal (90–125 mL/min/1.73 m^2) (n = 3) eGFR, matched as above, were also identified to serve as comparison with the HIV-infected subjects. A flow chart of the subject identification is shown in Supporting Information 2. The study protocol was approved by the UMMC Medical Research Ethics Committee (MREC) review board (MEC 20151–937). Informed written consent was obtained from all participants prior to sample collection.

2.2. Two-dimensional gel electrophoresis (2DE) and image analysis

A two-dimensional gel electrophoresis (2DE) experiment was performed on eight HIV-infected non-proteinuric CKD patients and their matched controls, using a previously described protocol with slight modification [14]. Briefly, for each sample, 4.5 μL plasma was added to 13.5 μL of sample buffer (9 M Urea, 60 mM DTT, 2% IPG buffer, 0.5% Triton X-100) to incubate for 30 min at room temperature. The sample mixture was then resuspended in 182 μL of rehydration solution (8 M Urea, 0.5% IPG buffer, 0.5% Triton X-100, 12 mM DTT, Orange G) for another 30 min at room temperature. IPG Immobiline DryStrips pH 4–7, 11 cm (GE Healthcare, Sweden) were rehydrated with samples overnight at room temperature. Isoelectric focusing on Multiphor II (GE Healthcare) was started at 300 V for 30 min and increased to 3500 V at 15 kV/h for approximately 5 h. The focused strips were sequentially incubated in SDS equilibration buffer (6 M Urea, 75 mM Tris-HCl pH 8.8, 29.3% Glycerol, 2% SDS) with 1% DTT and 4.5% iodoacetamide, for 15 min, respectively. The equilibrated strips were subjected to electrophoresis on 8–18% gradient SDS-polyacrylamide gels at 40 mA per gel in a SE 600 Ruby set (GE Healthcare). The gels were silver stained and scanned with ImageScanner III (GE Healthcare). The differences in percentage of spot volumes between the two groups were analyzed using ImageMaster™ 2D Platinum v7.0 (GE Healthcare). A p-value of < 0.05 was considered significant.

2.3. Trypsin digestion and mass spectrometry

Protein identification of spots of interest was performed using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry (MS) as previously reported with slight modification [14]. Fresh gels were run and subjected to MS-compatible silver staining. The protein spots were manually excised from the 2DE gels and destained with potassium ferricyanide (III). Gel plugs were sequentially incubated in 100 mM ammonium bicarbonate solution containing 10 mM DTT for 30 min at 60 °C for protein reduction, followed by 55 mM iodoacetamide for 20 min in the dark for alkylation, respectively. Gel plugs were washed in 100 mM ammonium bicarbonate containing 50% ACN and then replaced with 100% ACN. After freeze drying, the gel plugs were digested with 20 ng/L trypsin in 40 mM ammonium bicarbonate containing 10% ACN at 37 °C overnight, followed by peptide extraction using 50% and 100% ACN. The extracts were lyophilized, reconstituted in 0.1% formic acid, and desalted using C_{18} reversed phase media ZipTip columns (Millipore, USA). The peptide mixture was then mixed with 5 mg/mL alpha-cyano-4-hydroxycinnamic acid in a 1:1 ratio, spotted onto OptiToF MALDI 384-well plate and analyzed by MALDI-TOF/TOF 5800 mass spectrometer (Applied Biosystem/MDS Sciex, USA). Protein identification based on the mass spectra was performed using the MASCOT search engine [15] and searched against *Homo sapiens* entries in the Swiss-Prot database (Last updated: September 2017, 555,594 sequences). The selection parameters were: enzyme - trypsin, missed cleavage - 1, variable modification - 2; (i) carbamidomethylation of cysteine, and (ii) oxidation of methionine, peptide tolerance - 100 ppm, MS/MS fragment ion mass tolerance - 0.2 Da, and inclusion of monoisotopic masses only.

2.4. Verification of altered protein abundance

Western blotting was first performed on eight HIV-infected non-proteinuric CKD subjects and their matched controls, initially examined in the 2DE experiments, to verify the altered protein abundance observed in the 2DE analysis. Subsequently, we analyzed protein abundance in the five proteinuric subjects and the three HIV-uninfected subjects with low eGFR, and their respective matched controls. A total of 3 μg of protein for each sample, determined using Bradford assay [16], was separated on 10% SDS polyacrylamide gel and transferred onto PVDF membranes (0.2 μm, PALL Life Sciences, USA) using a Mini

Trans-Blot® Electrophoretic Transfer Cell (Bio-Rad, USA) for 1 h at 100 V. Membranes were stained for total protein using MemCode™ Reversible Protein Stain Kit (Thermo Scientific, USA), dried and scanned to be used as a loading control. The signal of MemCode™ staining instead of protein signals of commonly used housekeeping genes, such as transferrin, was used as a loading control. The former stains all protein molecules in a non-specific manner and majority of the protein molecules were not altered in the conditions we tested, hence providing a better control for loading. After removal of MemCode™ staining, the membranes were blocked with 1% BSA in 1 × TBST (25 mM Tris, 500 mM NaCl, 0.05% Tween, pH 7.5) for 30 min, washed 3 × 5 min with 1 × TBST, and incubated with primary rabbit antibody overnight at 4 °C. After washing with 1 × TBST thrice, the membranes were incubated for 1 h with horseradish peroxidase conjugated goat anti-rabbit antibody. The membranes were washed again with 1 × TBST and developed using 3, 3′-diaminobenzidine (Thermofisher, USA). MemCode™ signal and protein band intensities were obtained using the GelAnalyzer Software (www.gelanalyzer.com). Antibodies used are listed in Supporting Information 3.

2.5. Statistical analysis

Values are expressed as median (interquartile range), unless stated otherwise. Statistical analysis for Western blot data was performed using a Prism 8 software (GraphPad Software, USA). Mann-Whitney *U* test was used to analyze Western blot data and a *p*-value of < 0.05 was considered significant.

3. Results

3.1. Demographics and clinical characteristics

Among the 13 HIV-infected subjects with CKD, eight had no or had trace proteinuria (categorized as non-proteinuric CKD) while the other five had proteinuria (categorized as proteinuric CKD). On the other hand, all three HIV-uninfected subjects with impaired renal function were non-proteinuric. Demographics and clinical characteristics of the HIV-infected and HIV-uninfected subjects are summarized in Table 1.

Among the 13 HIV-infected patients with CKD, five patients had a known history of being prescribed with TDF-based regimen, of which only one patient was still taking TDF at the point of sample collection. On the other hand, of the 13 HIV-infected patients without CKD that served as control subjects, 12 were given TDF-based regimen, of which 10 was still taking TDF at the point of sample collection; one patient did not have a complete medication history record.

3.2. 2DE and MALDI-TOF/TOF analysis

2DE experiments were performed on plasma samples of eight HIV-infected non-proteinuric CKD subjects with an eGFR < 60 mL/min/1.73 m^2 and their respective controls. Representative gel images of the 2DE profiles of a non-CKD subject and a CKD subject are shown in Fig. 1A and B, respectively. By using ImageMaster™ software, spot detection performed on all the eight pairs of gel images resulted in the detection of approximately 500 protein spots per gel. Automated spot matching between images was performed and manually examined to ensure accurate spot detection and matching across all 16 gel images. Protein abundance for each detected spot was represented as percentage of spot volume. From the manual inspection of 63 well-resolved protein spots, eight were found to be significantly different in relative abundance between the two groups, with a fold change of ≥ 1.5 and a *p*-value of < 0.05 (Fig. 1C).

For the purpose of identifying the eight shortlisted protein spots of interest, 2DE experiment was repeated on one non-CKD sample with four technical replicates. The protein spots of interest from all the four gels were manually excised for MALDI-TOF/TOF protein identification. Proteins that were successfully identified were alpha-1-microglobulin/bikunin precursor (A1M), serum albumin (ALB), zinc-alpha-2-glycoprotein (AZGP1), haptoglobin (HP), and retinol binding protein (RBP4). Table 2 summarizes information of the successfully identified protein spots including the fold changes from the 2DE experiments, number of peptide matches, and the sequence coverage. Spot 1 was excluded from subsequent analysis due to insufficient data for identification of the protein. The abundance of A1M (spot 9), AZGP1 (spots 24 and 25), HP (spots 21 and 60), and RBP4 (spots 5) were found to be higher in the CKD group with fold changes ranging from 1.73 to 2.81; ALB (spot 10) demonstrated lower abundance in the CKD group with a fold change of 0.68, namely 1.5-fold lower in the CKD group compared to the non-CKD group.

3.3. Verification of altered protein abundance

Based on the MS results, abundance of four proteins (A1M, AZGP1, HP, and RBP4) in the plasma of all HIV-infected patients with and without CKD were examined with Western blotting. Representative gel images are shown in Supporting Information 4.

For the eight non-proteinuric CKD patients, the Western blot analysis detected a significantly higher abundance of A1M and HP compared to their controls, with a fold change of 1.56 and 1.68, respectively (Fig. 2). Interestingly, plasma levels of all four proteins from the patients diagnosed of HIVAN and Fanconi syndrome were both detected at levels lower than the mean. When the HIVAN and Fanconi cases were removed, the differences remained statistically significant with fold

Table 1
Subject demographics and clinical data.

Demographic	HIV-infected subjects		HIV-uninfected subjects	
	CKD (n = 13)	Non-CKD (n = 13)	Low eGFR (n = 3)	Normal eGFR (n = 3)
Age[a], years	53.00 (45.08–59.28)	49.39 (45.91–57.19)	64.98 (61.54–77.11)	70.51 (63.92–71.44)
Gender[b]				
Male	10	10	2	2
Female	3	3	1	1
eGFR[a], mL/min/1.73 m^2	52.00 (44.50–57.00)	102.90 (96.70–107.30)	55.90 (47.80–56.50)	90.70 (89.90–120.50)
Proteinuria[b]				
Non-proteinuric	8	13	3	3
Proteinuric	5	–	–	–
Comorbidities[b]				
Hypertension	10	10	3	3
Diabetes mellitus	4	4	2	2

[a] Median (interquartile range)
[b] Number of subjects

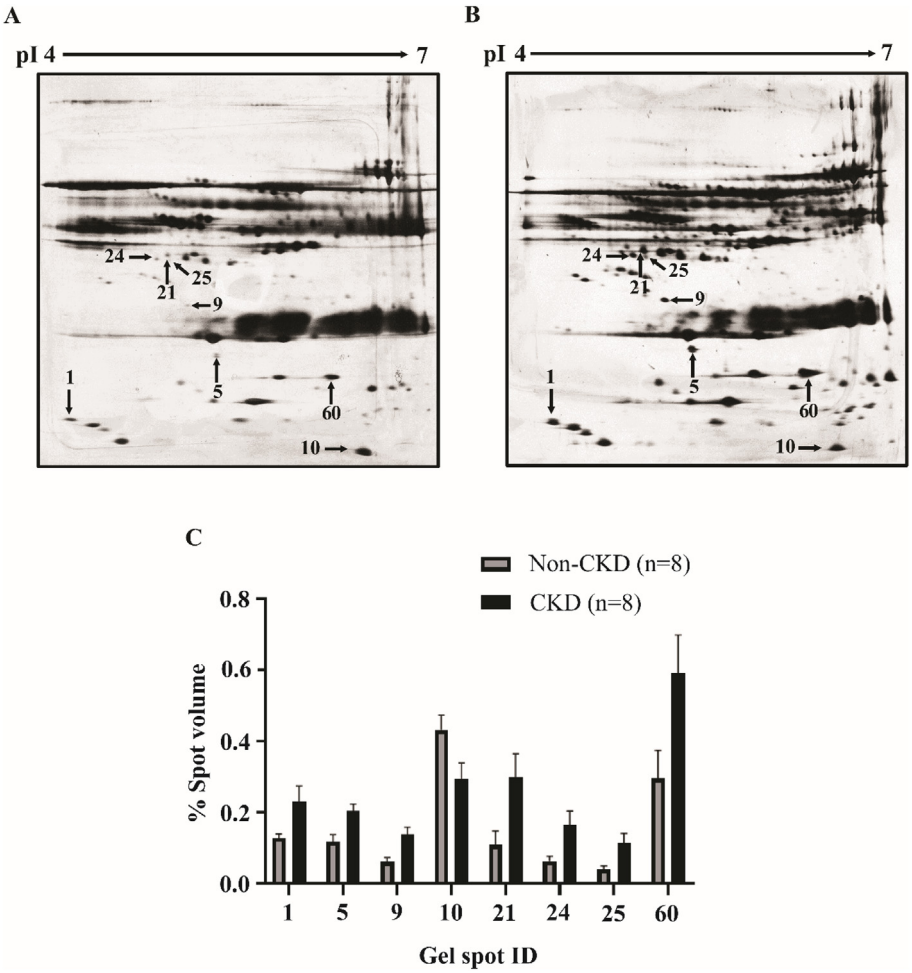

Fig. 1. 2DE analysis of plasma proteins of HIV-infected individuals with and without CKD. 2DE experiments were performed on eight patients with non-proteinuric CKD and their matched controls. Shown here are representative gel images of a (A) non-CKD and (B) CKD subject, respectively. (C) A total of eight protein spots showed statistically significant ($p < 0.05$) differences of ≥1.50-fold in percentage of spot volume, respectively. Data is presented as mean ± S.E.M. Note: Protein spots were arbitrarily numbered. pI, isoelectric point.

Table 2
Protein identification of gel spots by MALDI-TOF/TOF.

Gel spot no.	Swiss-Prot accession number	Protein	No. of peptides matched[a]	Sequence coverage[b] (%)	Fold change[c]
5	P02753	RBP4	6	45	1.73
9	P02760	A1M	5	19	2.23
10	P02768	ALB	1	1	0.68
21	P00738	HP	7	15	2.72
24	P25311	AZGP1	9	29	2.61
25	P25311	AZGP1	5	15	2.81
60	P00738	HP	5	11	2.00

[a] No. of peptides matched: Number of peptides that corresponds to the observed MS spectra for each protein spot analyzed.
[b] Sequence coverage: Percentage of the protein sequence that is covered by the identified peptides.
[c] Fold change: Ratio between the average percentages of spot volumes of eight subjects with non-proteinuric CKD versus their matched non-CKD controls based on 2DE image analysis.

changes of 1.84, 1.43, 1.82, and 1.37 for A1M, AZGP1, HP, and RBP4, respectively (Supporting Information 5).

In five proteinuric CKD patients, A1M, AZGP1 and RBP4, but not HP, showed a significantly higher protein abundance compared to the non-CKD group, with a fold change of 1.80, 1.41, and 1.80, respectively (Fig. 3). Western blot experiment was also performed for three HIV-uninfected individuals with low eGFR and their matched controls, for A1M, AZGP1, HP, and RBP4. There was no statistically significant difference seen in the four proteins examined (Fig. 4).

4. Discussion

In this study, we explored the difference of plasma protein profiles between HIV-infected subjects, with and without CKD. The subjects were matched for age, gender, ethnicity, and medical history of hypertension and diabetes mellitus, as these characteristics are known to affect estimation of glomerular filtration rate [2,3]. While the use of anti-retroviral agents, particularly TDF, is also associated with development and progression of CKD [3], the medication history could not be perfectly matched in this retrospective study. This is primarily because HIV patients presented with CKD would not have be prescribed a TDF-based regimen while those who developed CKD would be switched to a non-TDF-based regimen, in view of the nephrotoxic effect of TDF [4].

We found a higher level of plasma A1M in HIV-infected subjects with CKD compared to those who do not have CKD, regardless of their proteinuria status. Encoded by the alpha-1-microglobulin/bikunin precursor (AMBP) gene, A1M is a lipocalin protein that is primarily synthesized in the human liver and secreted into the blood circulation [17]. It is commonly termed as a tissue housekeeping protein with functions such as extracellular heme clearance and free radical

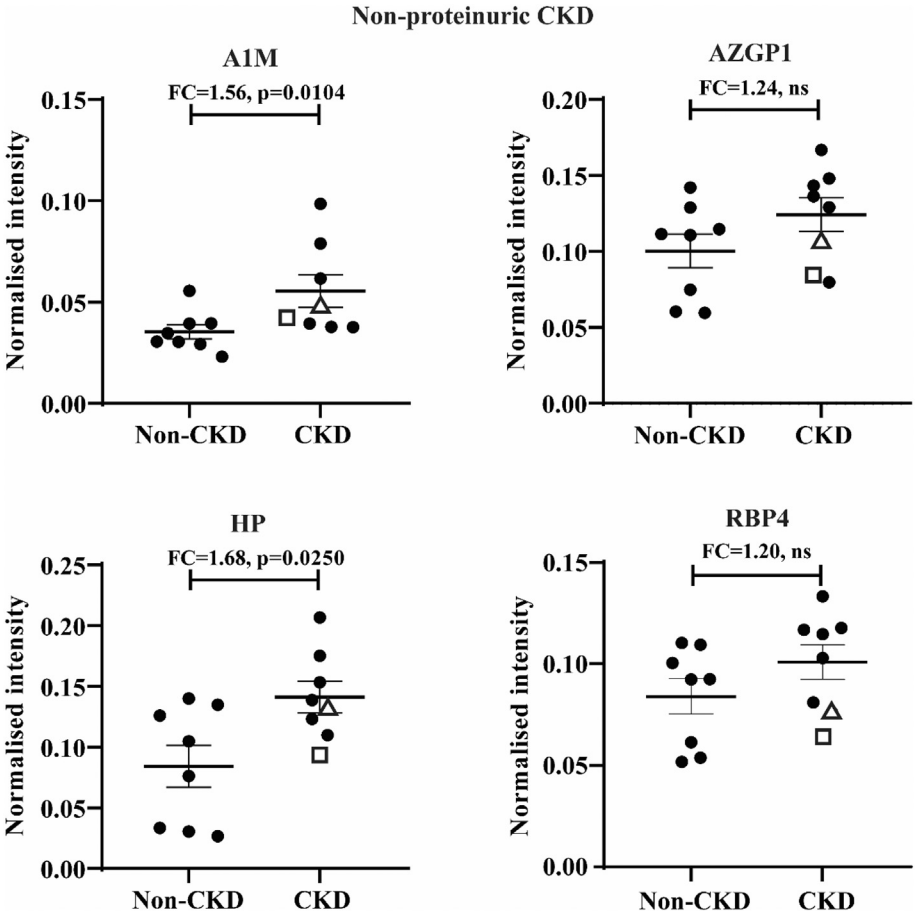

Fig. 2. Fold change for A1M, AZGP1, HP, and RBP4 in HIV-infected patients with non-proteinuric CKD. Data is presented as mean ± S.E.M; the square (□) and triangle (Δ) shape show data for the HIVAN patient and Fanconi syndrome patient, respectively. FC, fold change; ns, not significant.

scavenging. Given its low molecular weight (about 26 kDa), A1M proteins get filtered through the glomeruli easily, of which most of them are reabsorbed from the renal proximal tubules back into the circulation and the remaining are excreted into the urine [17]. It was reported that an increase in serum and urinary A1M coincides with an increase in serum creatinine and blood urea nitrogen (BUN), two common waste products that their accumulation in the body signifies impaired kidney function [18]. In a systematic review, Penders and Delanghe suggested urinary A1M to be used for the diagnosis and monitoring of renal and urinary tract disorders [19], although a derangement of urinary A1M was also found in non-renal pathology [20]. Interestingly, various groups have demonstrated urinary A1M to be a useful early marker that indicates renal tubular toxicity in HIV-infected individuals [21,22]. Here, we extend this observation to its usefulness as a biomarker of kidney function in the blood.

We have also found AZGP1, along with A1M, to be of higher abundance in the HIV-infected proteinuric CKD patients. AZGP1, a single chain polypeptide, is an adipokine that is involved in lipid metabolism [23]. Apart from being expressed in the adipocytes, AZGP1 protein was also found in multiple organs in human, including the renal proximal and the distal tubules [24]. In keeping with its wide tissue distribution, it is believed that AZGP1 is a multifunctional protein that is involved in various biological processes, including cell proliferation, cell adhesion, and cancer [24]. Similar to A1M, AZGP1 protein have small molecular mass (about 34 kDa) and thus are filtered freely through the glomerulus, after which they are reabsorbed from the kidney tubular cells [25]. In both acute and chronic kidney disease, there is evidence of elevated circulating AZGP1 in the serum [26]. Also, elevated urinary AZGP1 is associated with post-operative acute kidney injury [27] and in diabetic nephropathy [28]. Of note, in an experimental model of kidney fibrosis, AZGP1 was found to play a renoprotective role by preventing kidney fibrosis [29]. In treated HIV patients, lower circulating AZGP1 levels were observed compared to healthy uninfected subjects [30].

In our cohort of HIV-infected non-proteinuric CKD patients, HP, in addition to A1M, was found to be of higher abundance compared to those without CKD. HP is primarily synthesized in the liver but also expressed in other tissues such as lung and adipose tissue [31]. The main function of HP is the binding to free plasma hemoglobin (Hb) to form a large molecular Hb:Hp complex (> 150 kDa), thus preventing the damaging effects of Hb translocation into the kidneys [32]. In mouse models of different acute kidney injury, the expression levels of HP was found to be increased in the plasma, urine, and specifically in the proximal tubules [33]. In humans, urinary HP was reported as a good predictor of rapid renal function decline in patients with type 2 diabetes [34]. Notably, HIV-infected children with renal injury were shown to have elevated urinary HP in comparison to HIV-infected children without kidney dysfunction [11]. The role of HP in the progression of CKD in HIV infection is yet to be understood.

In addition to AZGP1, HIV-infected CKD patients who had proteinuria also had higher abundance of plasma RBP4. RBP4 is a 21 kDa plasma protein that is mainly expressed in the liver and in adipocytes [35]. RBP4 is secreted into the circulation as a complex with retinol and transthyretin. Once retinol is released to the target cells, the remaining RBP4-transthyretin can be filtered through the glomerulus and then reabsorbed in the proximal tubules for catabolism. In HIV-infected children with biopsy-proven renal diseases, elevated urinary RBP4 was observed even in those with trace proteinuria, suggesting the use of

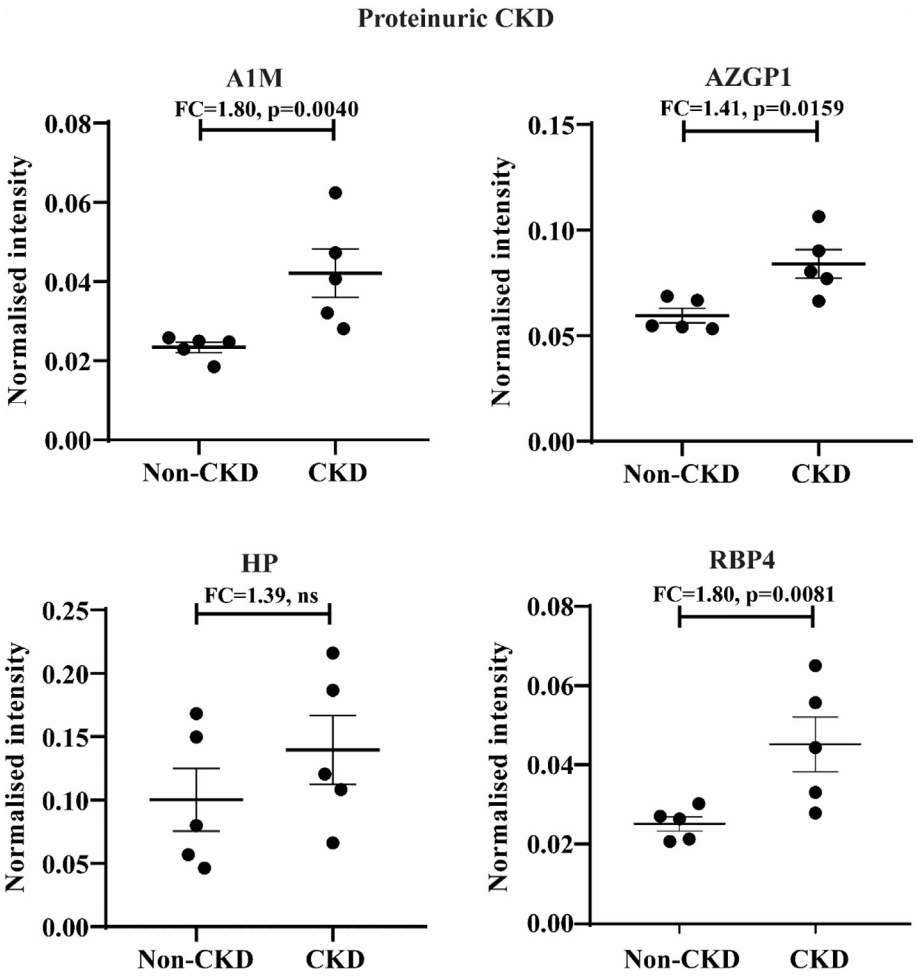

Fig. 3. Fold change for A1M, AZGP1, HP, and RBP4 in HIV-infected patients with proteinuric CKD. Data is presented as mean ± S.E.M. FC, fold change; ns, not significant.

RBP4 as an indication for early tubular damage [11]. Studies have also proposed the use of urinary RBP4:creatinine ratios in HIV-infected patients as a marker of tenofovir-associated tubular toxicity and Fanconi syndrome [36].

It is noteworthy that A1M, AZGP1, and RBP4 are of small molecular mass that allows them to be filtered freely through the glomeruli before being reabsorbed from the proximal renal tubules. Such property has made these proteins to be potentially useful as biomarkers of kidney impairment. A higher excretion rate of these proteins into the urine in HIV patients with kidney impairment, either as a result of leaky glomeruli or defected renal tubular reabsorption mechanism, would in theory lead to lower circulating levels in the plasma. In our study subjects, these proteins and HP were found to be elevated in the plasma. It remains to be elucidated whether this is due to a higher protein synthesis rate during the course of CKD that had somehow saturated the excretion mechanism in the kidney hence leading to accumulation in the body. We note that both patients with HIVAN and Fanconi syndrome, respectively, had lower than the mean plasma levels of all four proteins examined. More patients who share the same renal pathology are required for further investigations. It is of interest that in the HIV-uninfected group, who were of the older age range, no difference was observed in the plasma levels of the four proteins examined in relation to renal function, implying a potentially unique pathology of kidney damage in treated HIV-infected individuals. However, increased circulating levels of these proteins were detected in other diseases; for example A1M in preeclampsia [17] and HP in Alzheimer's disease [37]. Therefore, using these proteins as individual markers of renal function may not be feasible but could be combined to form a biomarker panel. It should also be acknowledged that we had a relatively smaller number of uninfected subjects examined and these results need to be confirmed in a larger cohort.

In summary, we propose to further validate the findings of this study in a prospective longitudinal study involving larger number of HIV-infected and -uninfected individuals, with and without CKD, to ascertain their value as biomarkers of kidney impairment. It would also be of great interest to determine the renal distribution of these protein in biopsied kidney samples of HIV-infected patients during the course of CKD development and progression, as well as in experimental animal models of CKD in the background of HIV infection to further elucidate the pathomechanisms of derangement of these proteins in the circulation.

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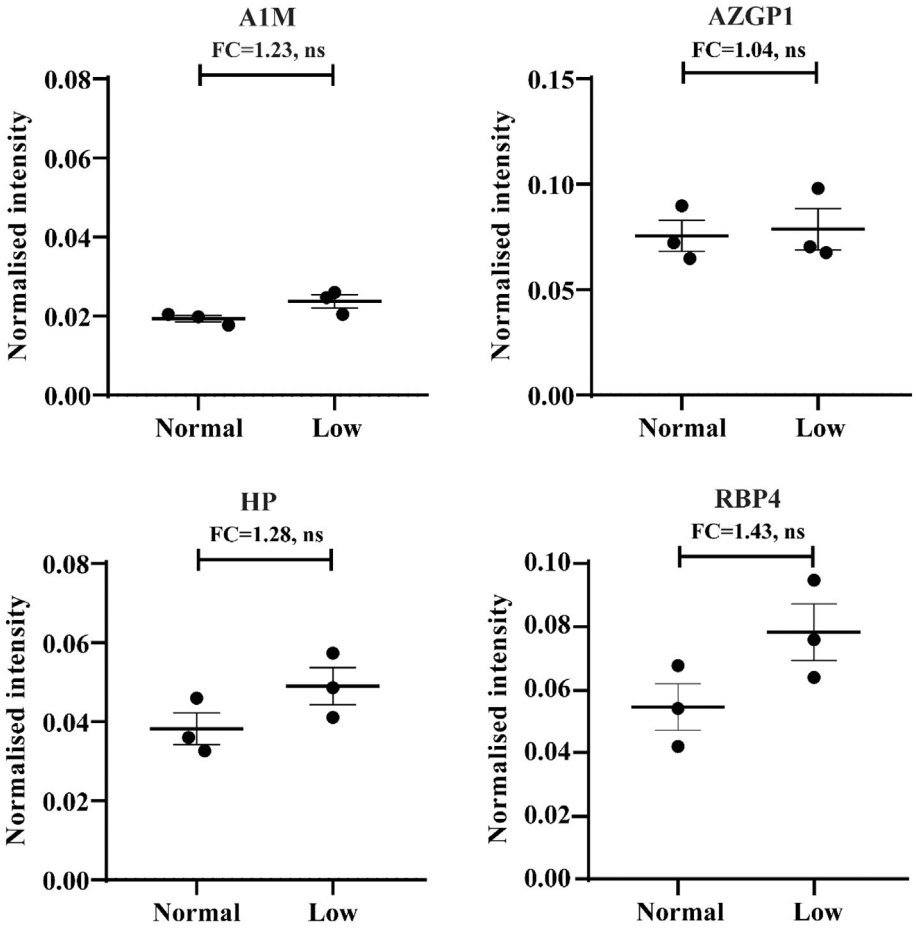

Fig. 4. Fold change for A1M, AZGP1, HP, and RBP4 in HIV-uninfected individuals.
Western blot was performed on HIV-uninfected subjects with eGFR rates < 60 mL/min/1.73 m^2 (Low) and 90–125 mL/min/1.73 m^2 (Normal). Data is presented as mean ± S.E.M. FC, fold change; ns, not significant.

Declarations of interest

None.

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