

Peptides in Plasma, Urine, and Dialysate: Toward Unravelling Renal Peptide Handling

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Purpose: The peptidomes of spent hemodialysate, urine, and plasma are investigated, to shed light on peptide handling in the kidney.

Experimental Design: Fifteen plasma, 15 urine, and 13 spent hemodialysate samples are collected from age- and sex-matched subjects with chronic kidney disease. Peptide identification and quantification are performed with capillary electrophoresis-coupled mass spectrometry.

Results: A total of 6278 urinary peptides, 1743 plasma peptides, and 1727 peptides from spent hemodialysate are detected. Of these, sequences can be assigned to 1580, 419, and 352 peptides, respectively. A strong correlation in peptide abundance between urine and spent hemodialysate ($p = 3 \times 10^{-21}$, $Rho = 0.52$), a moderately strong correlation between spent hemodialysate and plasma ($p = 4.5 \times 10^{-5}$, $Rho = 0.30$), and no significant correlation between urine and plasma ($p = 0.11$, $Rho = 0.094$) are found. Collagen and fibrinogen alpha peptides are highly abundant in all three body fluids. In spent hemodialysate, thymosin $\beta 4$ is one of the most abundant peptides, which is shown to be negatively associated with the estimated glomerular filtration rate ($Rho = -0.39$, p -value = 3.9×10^{-81}).

Conclusion and Clinical Relevance: The correlation of peptide abundance in these three body fluids is lower than expected, supporting the hypothesis that tubular reabsorption has a major impact on urinary peptide content. Further investigation of thymosin $\beta 4$ in hemodialysis is thus warranted.

1. Introduction

Proteins are the fundamental building blocks of life and the functional unit for biochemical reactions. Pathological changes in protein structure, expression level, or activity can lead to disease(s). Based on this theorem, diseases can be studied via the investigation of proteomics/peptidomics changes,^[1] with the help of recent advances in proteomics and peptidomics.^[2,3] Biofluids such as urine, plasma, and spent hemodialysate (a mixture of technical HD fluid and patient's ultrafiltrate) are rich sources for investigation,^[4,5] and highly relevant in kidney disease.^[6–8] In particular, peptides in spent hemodialysate may be valuable biomarkers to guide the treatment of dialysis patients: for example, deficiency of vitamin D-binding proteins in dialysis was reported to be associated with poor survival.^[9,10] Therefore, a better understanding of the flux of peptides may also help improving care for ESRD patients, to strike a balance between the clearance of toxic substances and retention of

beneficial molecules. Although “peptidomics” and “proteomics” are often used interchangeably, we refer our analysis as peptidomics because we study the undigested, naturally occurring endogenous peptides in the biofluids. Unlike proteomics, enzymatic/chemical digestion is typically not required for peptidomics analysis.^[11,12]

From a physiological point of view, peptides in these three biofluids should be, to a large extent, connected. Over 1700 L of blood, equivalent to one-fifth of the cardiac output, is filtered daily by the renal glomeruli via hydrostatic pressure. At a normal glomerular filtration rate (GFR) of 120 mL min^{-1} , this process forms around 170 L of ultrafiltrate. The ultrafiltrate then undergoes selective reabsorption in the renal tubule, so that the subsequent volume of urine drops to $\approx 1\text{--}2 \text{ L}$.^[13] The urine is collected in the calyx and via renal pelvis as the ureter enters the bladder, where it is stored for a few hours before voiding. In comparison, the spent hemodialysate is a simplified version of the ultrafiltrate fluid, produced using a dialysis membrane (an artificial kidney), with tubular reabsorption missing.^[14] It also lacks the secretory proteins from the kidney and urinary tract,^[15] and some middle-to-high molecular weight proteins may be depleted due to adsorption on dialysis membranes.^[16] The differences in peptide handling between hemodialysis and kidney are graphically illustrated in Figure 1.

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DOI: 10.1002/prca.202000029

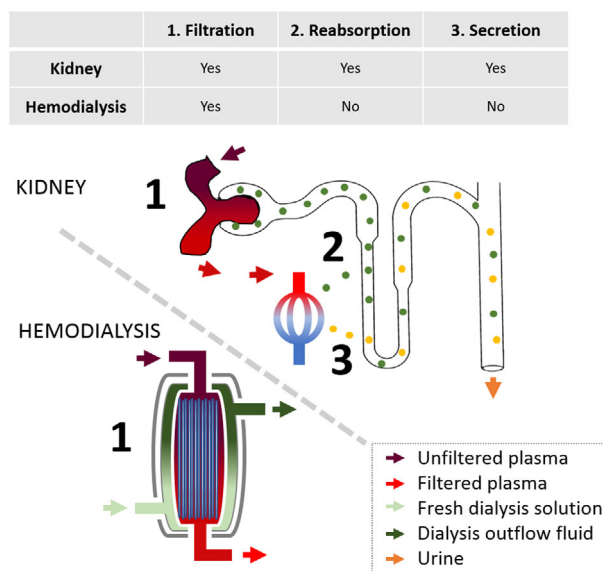


Figure 1. Representation of waste removal by kidney and hemodialysis. Filtration is present in both, while reabsorption and secretion are present only in the kidney.

The proteomic content of spent hemodialysate and its relationship with urine and blood are not well described. A study from Kaiser et al. suggests a minor overlap between the proteome of urine and spent hemodialysate,^[17] in agreement with the findings presented by the European Uremic Toxin Working Group (EUTox).^[18] A recent study by Magalhães et al. compared the peptidomics content of urine and plasma, revealing no correlation between the two peptidomes, except for collagen-based peptide fragments where a more pronounced overlap was detectable. The authors suggest that selective tubular reabsorption could account for the difference in the resulting peptidomes.^[19] Pedrini et al. identified 277 proteins from spent hemodialysate, among the most abundant are those with known uremic effect, such as complement factor D, β 2-microglobulin, retinol-binding protein 4, and myoglobin. However, the authors only assessed the tryptic peptides, but not the endogenous.^[20] Our exploratory study was based on the hypothesis that hemodialysis replaces glomerular filtration and aims at gaining first insight into the comparative distribution of the peptidome in these body fluids. This information may help to better understand the processes of filtration and reabsorption in the kidney, so that a renal replacement regime that better mimics the functionality of a kidney could be designed.

2. Results

2.1. Peptidome Profile of Spent Hemodialysate

In 15 samples, we detected a total of 1727 unique peptides (on average 755 peptides per sample) and obtained sequence information of 352 (20.4%) from them. The sequenced peptides covered 55.4% of the total detected peptide signal. The 20 most abundant peptides from spent hemodialysate are listed in Table S2A, Supporting Information. These were β 2-

Clinical Relevance

Hemodialysis is an effective way of uremic toxin removal, to compensate for the loss of kidney function in patients with end-stage chronic kidney disease. However, it is also associated with high costs and increased mortality, largely due to uremic effect. We investigated the peptidomes of three biofluids that are relevant to the kidney: spent hemodialysate, urine, and plasma, using capillary-electrophoresis-coupled mass spectrometry. Through a comparative analysis of the peptidomics profiles, we aim at a better understanding of the flux of peptides that may help improving care for ESRD patients, to strike a balance between the clearance of toxic substances and retention of beneficial molecules.

microglobulin, thymosin β 4, and fragments from fibrinogen α , collagen type I, and III, and from serum amyloid A-1.

When grouping the peptides according to their parental proteins/peptides, β 2-microglobulin accounted for the strongest combined signal (3 peptides, 51.8% of the total peptide signal), followed by thymosin β 4 (5 peptides, 24.4%), collagen alpha-1(I) (COL1A1) (123 peptides, 8.2%), and fibrinogen alpha (12 peptides, 5.8%). We identified five albumin fragments, which accounted for 0.06% of the total peptide signal. No fragments from uromodulin were detected (Table 1A).

2.2. Urine Peptidome

On average 1131 peptide signals were detected per sample. When combined, a total of 6278 urinary peptides were detected, 1580 (25.2%) of these could be sequenced, accounting for 74.3% of the total detected signal. As listed in Table 2B, Supporting Information, fragments of albumin were the most abundant peptides in the urine of CKD patients. Other abundant peptides were derived from α 1-antitrypsin, COL1A1, fibrinogen α , and β 2-microglobulin. As presented in Table 1B, albumin (49 fragments) alone was responsible for 45.7% of the total peptide signal, followed by α 1-antitrypsin (79 fragments, 14.4%), COL1A1 (319 fragments, 11.7%), and fibrinogen α (30 fragments, 4.9%). Other prominent peptides included β 2-microglobulin (3.9%) and uromodulin (1.8%).

2.3. Plasma Peptidome

A total of 1743 unique endogenous peptides were detected in 15 plasma samples. Of these, we could sequence 419 (24.0%) peptides, which accounted for 29.7% of the total detected peptide signal. The 20 most abundant peptides are listed in Table 2C, Supporting Information. The proteins of origin of these peptides are more heterogeneous than the results obtained from urine or spent dialysate. We detected 18.8% COL1A1 (113 fragments), 16.5% fibrinogen alpha chain (20 fragments), and nine fragments from α 1-antitrypsin (0.7% of the total signal), and six from thymosin β 4 (2.2%; Table 1C).

Table 1. Distribution of sequenced peptides from A) Spent hemodialysate, B) urine, and C) plasma samples from CKD patients and D) urine samples of normal albuminuria individuals. Peptides are sorted accounting to frequency. The relative abundance of peptide(s) (%) was calculated by Mean abundance $\times$ 100/Abundance of all peptides in a given cohort. Only peptides that were among the four most abundant peptides in at least one body fluid are presented. COL1A1: collagen alpha-1(I) chain.

	Number of identified peptides	Relative abundance [%]
A) In Spent hemodialysate (CKD)		
COL1A1	123	8.2
Fibrinogen alpha chain	12	5.8
Albumin	5	0.06
Thymosin beta-4	5	24.4
Alpha-1-antitrypsin	3	0.03
Beta-2-Microglobulin	3	51.8
Uromodulin	0	0
Others	201	10.0
B) In urine (CKD)		
COL1A1	319	11.7
Alpha-1-antitrypsin	79	14.4
Beta-2-Microglobulin	53	3.9
Albumin	49	45.7
Fibrinogen alpha chain	30	4.9
Uromodulin	27	1.8
Thymosin beta-4	4	0.3
Others	1019	17.1
C) In plasma (CKD)		
COL1A1	113	18.8
Fibrinogen alpha chain	20	16.5
Beta-2-Microglobulin	10	0.2
Alpha-1-antitrypsin	9	0.7
Albumin	8	3.2
Thymosin beta-4	6	2.2
Uromodulin	2	0.2
Others	252	25.0
D) In urine (normal albuminuria)		
COL1A1	614	46.9
Alpha-1-antitrypsin	79	0.1
Beta-2-Microglobulin	69	0.004
Albumin	52	0.03
Fibrinogen alpha chain	51	4.7
Uromodulin	46	13.9
Thymosin beta-4	8	0.05
Others	2109	34.3

2.4. Comparisons of the Peptidome Profiles

We identified 161 common peptides in three body fluids, at the same time 37, 1161, and 110 peptides were unique in spent hemodialysate, urine, and plasma, respectively, in our analysis (Figure 2). We listed all sequenced peptides in all three body fluids with their rank by mean abundance in Table S3, Supporting Information. COL1A1-derived peptides were the most frequently detected peptides in all three body fluids. In spent hemodialysate

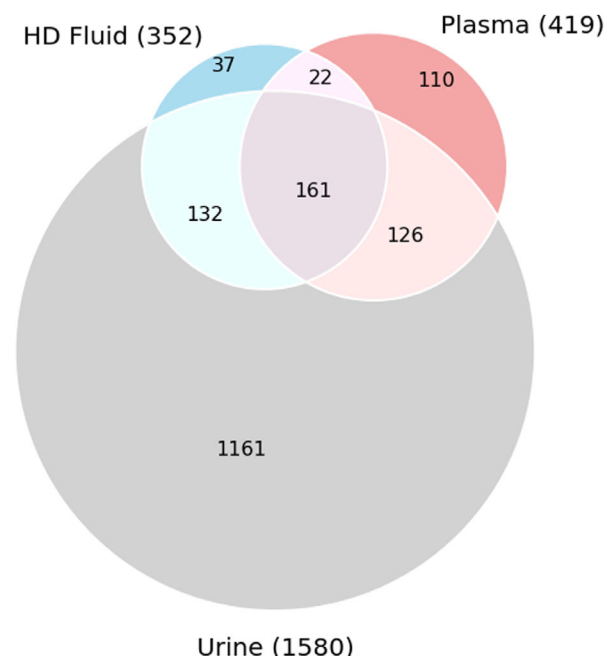


Figure 2. Comparison of the sequenced peptides in three body fluids. A total of 1161, 110, and 37 peptides were exclusively identified in urine, plasma, and spent hemodialysate, respectively. One hundred sixty-one peptides can be found in all three biofluids.

and plasma, fibrinogen alpha-derived peptides were the second most frequent; while in urine, the second most frequent was from α 1-antitrypsin. β 2-microglobulin and thymosin β 4 were the two most abundant peptides in Spent hemodialysate. These two peptides could also be detected in urine and plasma. The most abundant peptide in urine was from albumin, which was present but at a significantly lower level in plasma and spent hemodialysate. When examining the correlation of mean peptide abundance between the three peptidomes (Figure 3), a significant correlation between the peptidomes of Spent hemodialysate and urine ($R = 0.56$, p -value = 8.4×10^{-27}), and between spent hemodialysate and plasma with ($R = 0.28$, p -value = 8.4×10^{-5}) was detectable. However, no significant correlation ($R = 0.82$, p -value = 0.15) was found between plasma and urine peptidomes, in agreement with a previous study.^[19]

2.5. Reference to the Peptidomics from a Normal albuminuria Population

Since the urine samples investigated in this study were from patients with advanced-stage CKD (for comparability with patients on dialysis), the impact of proteinuria in the urine analysis could not be avoided and appeared obvious. To assess the potential influence of proteinuria, we compared the urinary peptidomics data with those from the general population, obtained from the FLEMENGHO study, which consists of 777 non-proteinuric urine samples.^[25] In this cohort, we identified 3154 urinary peptides. The peptides are listed in Table S3, Supporting Information, ranked according to mean abundance.

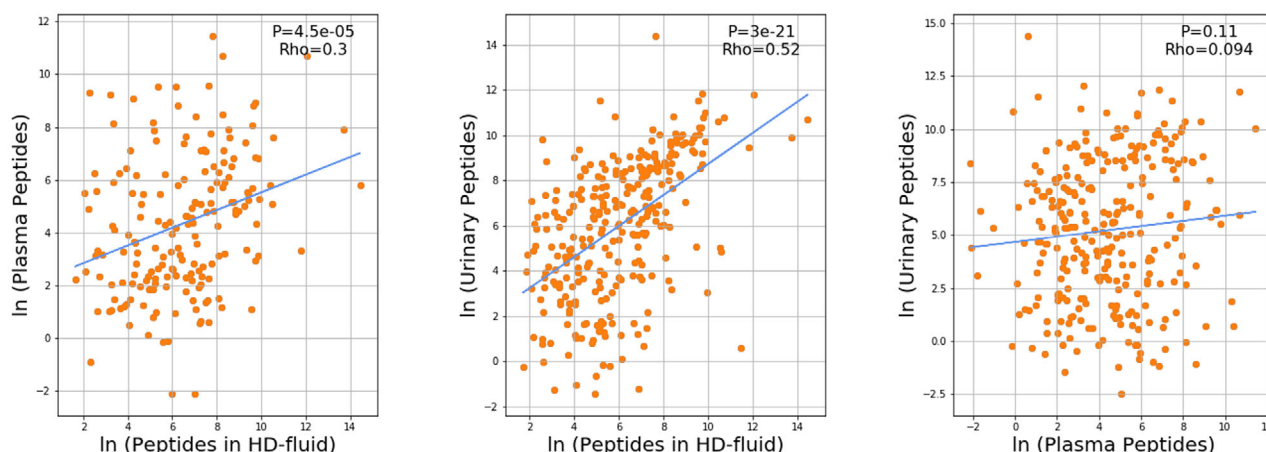


Figure 3. Correlation of the mean peptide abundance of overlapping peptides in spent hemodialysate, plasma, and urine. The peptide abundances were ln-transformed.

As listed in Table 2D, the peptides were predominantly from COL1A1 (614 fragments, 46.9% of total peptide signal) and uromodulin (46 fragments, 13.9%), while the signal from albumin plunged to 0.3% in comparison to the peptidome of CKD patients (45.7%). Among the 20 most abundant peptides (Table 2D, Supporting Information), fragments from albumin were no longer present. The most abundant peptide fragments came from uromodulin (3/20), fibrinogen α chain (1/20), collagen type III (4/20), and predominantly collagen type I (12/20), in very good agreement with previous studies.^[22]

The comparison of the 20 most abundant peptides between the normal albuminuria and CKD subjects is presented in Figure S1, Supporting Information. While the distribution of the 20 most abundant peptides from subjects with preserved kidney function remained relatively unchanged in the urine of the CKD patients (Figure S1, Supporting Information, right), the most abundant urinary peptides in CKD subjects were found only at highly reduced levels in the controls. These included eight albumin, four α 1-antitrypsin, two β 2-microglobulin, and two fibrinogen α chain peptide fragments (Figure S1, Supporting Information, left).

Most of the results obtained were as expected, however, the very high abundance of thymosin β 4 in spent hemodialysate was surprising and not reported previously. We therefore further examined the correlation between the abundance of thymosin β 4 and eGFR in 2289 patients from the urine proteome database.^[26] As shown in Figure 4, a highly significant negative association of thymosin β 4 abundance with eGFR ($R = -0.39$, p -value = 3.9×10^{-81}) was detected, which is exacerbated upon kidney failure.

3. Discussion

The urine and plasma peptidome of 15 CKD patients and spent hemodialysate from 13 patients were evaluated to shed light on the kidney peptide handling. Overall, we identified 352, 1580, and 419 peptide fragments in spent hemodialysate, urine, and plasma, respectively. The higher number of peptides in urine

is likely the result of a higher concentration due to tubular activity, reabsorption of water, and consequently increase in the concentration of compounds not being reabsorbed with similar efficiency.

To our knowledge, this is the first study to demonstrate a correlation between spent hemodialysate, urine, and plasma. Surprisingly, no similarity between urine and plasma could be found, although we found the expected similarity between urine and spent hemodialysate as well as between plasma and spent hemodialysate (Figure 3). Because both spent hemodialysate and urine are derived from plasma, the difference in correlation with plasma is likely due to differences in the mechanics of the kidney and dialysis membrane. As illustrated in Figure 1, reabsorption and secretion that are unique to the kidney but absent in hemodialysis could be the plausible sources of such difference. In addition, urinary peptides are subjected to the activity of kidney-specific proteases, not present in circulation, relevant for peptides in both plasma and spent hemodialysate.

We confirmed the presence of peptides in spent hemodialysate documented in the literature, including α 1-antitrypsin, albumin, apolipoprotein A-IV, β 2-microglobulin, fibrinogen α chain, gel-solin, insulin-like growth factor II, Ig kappa chain C region, osteopontin, and thymosin β 4.^[14] Among them, β 2-microglobulin and thymosin β 4 were the two most abundant peptides in spent hemodialysate. β 2-microglobulin, via interactions with other proteins, fosters the deposition of stable amyloid-like complexes in bones, tissues, vessels, and heart.^[27] Therefore, an elevated concentration of β 2-microglobulin in circulation may provoke deterioration of renal function in combination with adverse cardiovascular outcomes in CKD patients.

Thymosin β 4 was reported as potentially beneficial in CKD by regulating fibrosis and inflammation.^[28] We found a highly significant negative correlation between urinary thymosin β 4 and eGFR (Figure 4), in agreement with the high level of thymosin β 4 detected in spent hemodialysate. We could only detect a moderate level of thymosin β 4 in two of the 15 plasma samples that we analyzed. This is consistent with the literature description that concentration of thymosin β 4 in plasma was <1% of its

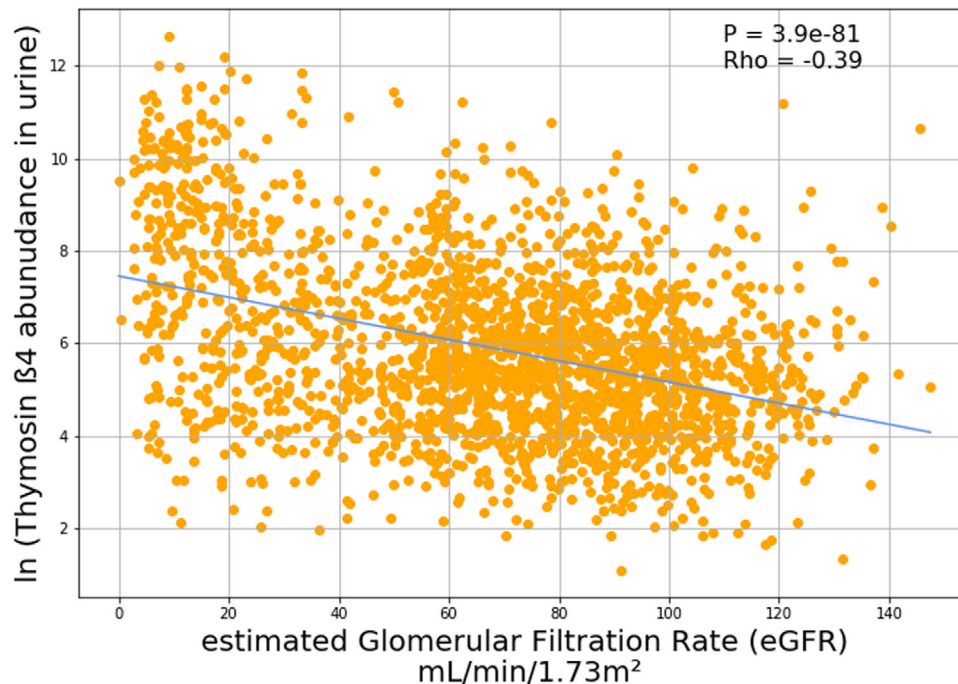


Figure 4. Correlation of the mean peptide abundance of one thymosin $\beta 4$ peptide in urine with eGFR of 2289 independent patients from the database. The peptide abundances were ln-transformed.

concentration in the whole blood.^[29] Studies in transgenic mice models suggested that endogenous thymosin $\beta 4$ is essential for kidney health, while a lack of endogenous thymosin $\beta 4$ worsens glomerular disease and angiotensin-II-induced renal injury in mice.^[30] Combining our finding that urinary thymosin $\beta 4$ is negatively associated with kidney function, it appears possible that increased urinary excretion of thymosin $\beta 4$, consequently a loss of thymosin $\beta 4$, is associated with CKD severity. Alternatively, the observed increase in urinary thymosin $\beta 4$ in advanced CKD may be the result of a compensatory protective response to kidney dysfunction, as an analogue to the elevated natriuretic peptide level during heart failure.^[31] The loss of thymosin $\beta 4$ from the circulation may be substantially higher in hemodialysis than in subjects with residual kidney function, which supports our postulation. Therefore, detailed studies comparing plasma and urinary/spent dialysate levels of thymosin $\beta 4$ in CKD are warranted to assess the potential its impact, especially in hemodialysis.

Another observation is the enrichment of albumin fragments in urine of the CKD patients, but not in plasma or spent hemodialysate. This is expected since albuminuria is a frequent feature of CKD.^[32] When investigating the urine peptidomes from a population-based cohort,^[25] the albumin fragments were no longer abundant (Table 1B,D). By comparing the most abundant urinary peptide fragments between the two groups (Figure S1, Supporting Information), we observed that most of the top peptides identified in CKD patients (mostly albumin and $\alpha 1$ -antitrypsin) were of low abundance in control subjects (Figure S1, Supporting Information, left). Albumin and $\alpha 1$ -antitrypsin are plasma-derived and typically present only at a very low level in the urine of healthy individuals.^[33] In contrast, top peptides (mainly collagen type I and III, as well as fibrinogen α chain) identified from the population-based cohort showed similar

relative abundance in CKD patients (Figure S1, Supporting Information, right). These data indicate that CKD may not affect the fragmentation of collagens and fibrinogens by proteases in the kidney.

In all three biofluids, collagens (mainly type I and III) and fibrinogens manifested high signal intensity. Based on the published data, collagens were proposed as biomarkers for diagnosis and prognosis early kidney and/or heart-related diseases,^[25,34] possibly indicating molecular changes in the extracellular matrix during fibrosis.^[7,35]

Our study has certain limitations. We could not obtain information about CKD etiologies of the HD patients, because their samples were collected anonymously. Association of peptides with CKD etiology was not the aim of the study, which is also not powered for this purpose. This study focuses on the inter-biofluid differences, we did not examine the possible intra-biofluid differences induced by medical treatment. Furthermore, the sample size is insufficient to assess the impact of medication, given the heterogeneity of the disease and the cohort, and the impact of multiple and diverse drugs applied in the patients. However, this does not have a major impact on the consistency of the results as the samples were matched in sex and age.

We are aware that the comparison between plasma and spent hemodialysate samples from the same HD patients would be most appropriate. Because of the lack of plasma samples from HD patients, we used plasma samples from advanced-stage CKD patients for consistency. The spent hemodialysate from HD patients may not be fully comparable to results obtained from CKD patients and healthy subjects. However, it is not possible to perform hemodialysis on individuals with preserved kidney function, due to evident ethical and medical reasons. We are aware that our current study is exploratory; the inclusion of more

samples, particularly from the HD patients, would be a logical next step to refine the existing results.

Another limitation is the moderate percentage of sequenced peptides (roughly 20% across the three body fluids). A major cause for this shortcoming appears to be the presence of unknown PTMs, prohibiting the correct matching of the spectra with the proteome database.^[36] Frequently, good spectrum quality still does not enable the assignment of sequence. Among others, fragment signals typically for glycosylation are present in many of the spectra where no sequence could be assigned.^[3,37] As a result, we are not aiming at improving peptide sequencing by investigating potential posttranslational modifications in more detail.

The gold standards to assess kidney function are urinary albumin level and eGFR, a derivative based on serum creatinine levels.^[38] Both of them are reliable biomarkers in determining the disease severity and therefore of substantial value in providing clinical guidance. However, both serum creatinine and albuminuria have shortcomings as biomarkers for early detection or guiding intervention in CKD. The elevation of serum creatinine is not conspicuous until a substantial fraction of renal function is lost. The loss can be as huge as 50%, likely due to the accompanying reduction in muscle mass as CKD progresses.^[39] As a result, the diagnosis of CKD based on eGFR is generally too late for effective intervention. Urinary albumin excretion has been proposed as a better predictor of accelerated renal function decline than eGFR.^[40] However, it is highly variable^[41] and lacks accuracy in assessing renal function decline: neither the presence nor the absence of albuminuria can detect or preclude CKD with certainty.^[42]

Despite the technological advances in clinical proteomics, to date, only a limited number of biomarkers based on MS are in use. The reason for this gap does not appear to be technical (e.g., sample handling or storage), but mostly a result of a lack of appropriate studies. Most of the MS-based biomarker studies focus on preliminary discovery in very small cohorts, but not on the biomarker validation/qualification, due to the significantly larger effort involved. As a result, the biomarkers are typically not brought forward to be employed in patient assessment.^[43] A change in attitude toward appropriately powered studies aiming at the validation of biomarkers has been proposed, which appears to be the solution to the limitations of existing biomarkers in CKD.^[44]

In summary, our study reports an in-depth characterization of three different peptidomes using the same MS platform. Comparing urine, plasma, and spent hemodialysate is expected to widen our understanding of the underlying molecular differences associated with endogenous peptide processing. Furthermore, this study reveals several interesting points: a) significant positive correlations between urine and plasma with HD fluid and no correlation between urine and plasma based on the overlapping peptide abundance, indicating selectivity of the tubular reabsorption via a yet unknown molecular mechanism; b) identification of thymosin β 4 as highly abundant in the HD fluid; and c) a significant negative correlation of urinary thymosin β 4 with eGFR. Future experimental studies are warranted to explore the biological relevance of thymosin β 4 in the context of CKD.

4. Experimental Section

Collection of Plasma, Urine, and HD Fluid Samples: HD fluid samples were collected from 13 hemodialysis patients from the filtration device. The plasma and urine samples were collected from 15 age and sex-matched patients. Their characteristics are reported in Table S1, Supporting Information.

The study was conducted fulfilling all laws on the protection of individuals being involved in medical research and accordance with the principles of the Declaration of Helsinki. All samples and data were obtained anonymized. The local ethics committee from Hannover Medical School (Hannover, Germany) approved the approach, employing anonymized samples and proteomics data (Ethical ID: 3596-2017).

Sample Preparation: All samples were analyzed individually, no pooling was performed. The performance of the procedures, including sample preparation and analyses, have been assessed in detail,^[21] and are continuously monitored based on a "standard urine sample."^[22] As a result, analysis of replicates appeared not required. In general, peptide degradation in urine is of minor concern, since digestion by endogenous proteases was complete after hours of storage in the bladder before voiding. The sample quality is controlled by the presence of known peptides in prespecified amounts.^[23] Degradation by, e.g., exogenous proteases due to contamination would fail to pass the quality control. This procedure has been applied and was found valid in >70 000 samples.^[21]

Urine and Plasma samples were prepared essentially as described previously.^[19] In short, 0.7 mL of the urine sample was diluted with 0.7 mL of 8 M urea, 1×10^{-2} M NH_4OH , and 0.02% SDS. Plasma samples were diluted by adding 0.4 mL H_2O to 0.3 mL plasma to a total volume of 0.7 mL.^[24] HD fluid samples were prepared without ultrafiltration. A volume of 2.5 mL of the HD fluid was directly applied onto a PD-10 desalting column eluted with 2.5 mL 1×10^{-2} M NH_4OH .

CE-MS and MS/MS Analysis and Data Processing: CE-MS analysis was performed as previously described^[21] in the same way for all samples. A P/ACEMDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA) was coupled with a Micro-TOFMS (BrukerDaltonics, Bremen, Germany). Acetonitrile (20%; Sigma-Aldrich, Taufkirchen, Germany) in HPLC-grade water (Roth, Karlsruhe, Germany) supplemented with 0.94% formic acid (Sigma-Aldrich) was used as running buffer. The electrospray ionization interface from Agilent Technologies (Palo Alto, CA) was set to a potential of -4.0 to -4.5 kV. Spectra were recorded over an m/z range of 350–3000 and accumulated every 3 s.^[21]

Statistical Analysis: Pearson correlation was used to examine the association of peptide abundances between the body fluids. A univariable linear regression model was used to assess the association between thymosin β 4 abundance and eGFR. In both analyses, the abundances were $\ln$ -transformed; samples with no peptide detected were omitted. The level of association was evaluated based on ρ (R) and p -values. p -Values < 0.05 were considered statistically significant. Python 3.7 was used to perform these analyses and to generate the corresponding graphs. Additional detailed information about the Experimental Section is presented in the Supporting Information. The original peptide abundance in samples are presented in Table S4, Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The work was supported by the CaReSyAn Project (Project ID: 764474) under the MSCA-ITN-2017-Innovative Training Networks, and by the European Uremic Toxin Work Group (EUTox).

Open access funding enabled and organized by Projekt DEAL.

Data Availability Statement

The mass-spectrometry proteomics data were deposited to the ProteomeXchange Consortium with an identification number 421225.

Conflict of Interest

T.H. and M.P. are employed by Mosaïques Diagnostics GmbH (MOS); H.M. is the co-founder of MOS; other authors declare no conflict of interest.

Keywords

hemodialysis fluid, peptidomes, plasma, urine

Received: April 3, 2020
Revised: June 11, 2020
Published online: July 23, 2020

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