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Urine Exosomal Ceruloplasmin a Potential Early Biomarker of Underlying Kidney Disease

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Abstract

Background: Previously we found that kidney tissue and urinary exosomes from patients of diabetic kidney disease (presenting with microalbuminuria) showed high levels of ceruloplasmin (CP). Because CP is an acute phase protein of kidney origin, it could be an early marker of many other kidney diseases. To investigate this hypothesis we first measured urine exosomal and kidney expression of CP in non-diabetic kidney disease patients (membranous nephropathy, focal segmental glomerulosclerosis, lupus nephritis and IgA nephropathy) followed by a longitudinal study in rat passive Heymann nephritis (PHN), a model of human membranous nephropathy.

Methods: Urinary exosomes were isolated from urine of chronic kidney disease patients (and rats) by differential centrifugation. The exosomal extracts were used for measuring CP using ELISA. Kidney expression of CP was evaluated by immune-staining biopsy tissues. Similar techniques were applied in rat PHN model to analyze urine exosomal and kidney CP. Rat PHN was produced by injection of anti-gp600 antiserum prepared previously.

Results: Ceruloplasmin level was found to be >10-20 times higher in CKD patient samples compared to control subjects. Consistent with the high CP levels in exosomes, we found high immune-reactive CP localized in tubules and collecting ducts of biopsies of CKD patients. In the PHN model urinary exosomal CP level was significantly higher prior to the onset of proteinuria. Early rise of urine exosomal CP, which preceded proteinuria, correlated with high immunoreactive CP found in rat kidneys at this time.

Conclusion: We propose that increase of urine exosomal CP is a signal for underlying kidney disease occurring prior to proteinuria, making it a potential urinary biomarker to diagnose early kidney disease of many different etiologies.

Keywords: Ceruloplasmin; Exosomes; Chronic kidney disease; Immunohistochemistry; Proteinuria; Heymann nephritis

Introduction

Urinary exosomes offer a novel compartment for study because they contain proteins and markers that are specific to the kidney [1-4]. Further, they are easy to isolate because urine remains uncontaminated by blood-borne exosomes as they are large-sized bodies that cannot pass through the glomerular barrier. Examining urinary exosomes for kidney disease markers may fulfill the much-sought-after promise of replacing tissue biopsies (which are invasive), with 'liquid biopsies' (which are non-invasive).

Previously we found that urinary exosomes from patients of early diabetic kidney disease (presenting with microalbuminuria) showed high levels of ceruloplasmin (CP) using enzyme immune-assay (ELISA) [3]. Further, high CP in urinary exosomes correlated with high immunoreactive CP found on biopsies of diabetic kidney disease patients (localized to tubules) [3], suggesting that urine exosomal CP is synthesized and derived from the kidney tissue. An interesting corollary of this finding is, because CP is an acute phase protein of kidney origin it could be an early marker of many other kidney diseases, as most kidney diseases likely begin with inflammation and/or other metabolic insults in the kidney tissue.

To investigate this hypothesis we first measured urine exosomal CP by ELISA in biopsy-proven patients with non-diabetic chronic kidney diseases (CKD), including membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS), lupus nephritis and IgA nephropathy. Subsequently we

histochemically stained these biopsies for CP to confirm their origin in the kidney, and validate the ELISA results. Finally, in order to explore whether the urine exosomal CP was an early marker of kidney disease, one that precedes proteinuria, we performed a longitudinal study in rat passive Heymann nephritis (PHN), an animal homologue of human membranous nephropathy. A similar longitudinal study in human patients which would take 3-4 years to complete is currently in progress. Based on the above, it was felt important to pursue this question in an animal model to put the hypothesis to an initial test. The choice of using PHN as the model to examine this question was based on 1) rapid onset of proteinuria in this model and 2) absence of a readily available animal model of other chronic kidney diseases.

Materials and Methods

Patients

The study was approved and conducted according to the guidelines of the Institutional Review Board of John H Stroger, Jr. Hospital of Cook County, Chicago IL. Urine samples were collected, prior to biopsy, in sterile 100 ml plastic bottles from biopsy-proven patients of membranous nephropathy (N=9), IgA nephropathy (N=7), lupus nephritis (N=10), focal segmental glomerulosclerosis (N=10), and normal controls (N=12). In addition urine samples were collected randomly from non-biopsy proven CKD stage 1 and 2 patients (N=15) attending renal clinic at this hospital. Basic demographic data including proteinuria, serum creatinine, and eGFR at time-of-visit are presented in (**Table 1**).

Induction of PHN in rats

Animal experiments were performed in the rat model of PHN, which mimics human membranous nephropathy. All experiments were conducted as per the guidelines and approval of the Institutional Animal Care Committee. Groups of Sprague-Dawley rats (250-300 g) (Harlan Laboratories, Indianapolis, IN USA) were injected intravenously with 1.0 mL of rabbit anti-gp 600 antiserum *via* the tail vein. Anti-gp 600 antiserum was prepared in rabbits as described previously [5]. Three days after the intravenous injection a booster dose of 1.0 mL of antiserum was administered intra-peritoneally. Control rats were injected with similar volumes of sterile phosphate (10 mM) buffered saline (125 mM NaCl) pH 7.4 (PBS) instead of antiserum at corresponding times. Twenty-four hour urine was collected using metabolic cages on day 0, week 1, week 2 and week 3, to monitor proteinuria and prepare exosomal extracts as described below. Urine was collected in a container containing protease inhibitor cocktail (Sigma-Aldridge, St Louis, MO) to inhibit endogenous protease activity. Few rats from the experimental and control groups were sacrificed at day 0, week 1, week 2 and week 3 to harvest kidneys for histology and histochemistry. Passive Heymann nephritis was confirmed by presence of dense and granular glomerular IgG deposits using histochemical staining.

Table 1: Clinical and demographic characteristics of study subjects.

Group (Number of subjects)	Age (years)	Gender (% Female)	Mean Proteinuria (g/g creatinine) (range)	Mean serum Creatinine (mg/dL) (range)	eGFR* (mL/min/1.7 M^2) (range)
MN*(9)	49.3 ± 5.3	67	6.4 (2.8-14.5)	1 (0.5-2.0)	41-135
IgAN*(7)	42.3 ± 5.3	57	4.3 (2.2-7.4)	3.1 (0.5-8.8)	8-132
Lupus nephritis (10)	30.9 ± 3.2	60	4.9 (1.6-9.9)	1.6 (0.6-5.6)	12-146
FSGS*(10)	41.4 ± 3.7	50	3.2 (0.2-9.4)	1.9 (0.4-4.0)	16-143
CKD*1 and 2 (16)	34.4 ± 3.3	25	1.6 (0.48-3.4)	1.2 (0.6-1.8)	84-146
Controls (12)	45.4 ± 6.1	30	<0.15	n.d.**	n.d.**

*eGFR=Estimated glomerular filtration rate by MDRD formula; MN = membranous nephropathy; IgAN=IgA nephropathy; FSGS=Focal segmental glomerulosclerosis; CKD=Chronic kidney disease; n.d.: not done

Preparation of exosomal extracts

Upon collection, urine (50-100 mL human or 10-20 mL rat) was immediately centrifuged at 1000 x g for 20 minutes at 4°C to remove cellular material, crystals and other debris. The supernatant was separated and treated with 60 mg/ml reducing agent dithiothreitol (DTT) (Sigma-Aldridge) to denature Tamm-Horsefall Protein (THP) and thereby inhibit the aggregation of THP (to avoid contaminating the exosomal fraction). Supernatants were stored at -85°C until further processed for preparation of exosomal extracts. Subsequently, urinary exosomes were isolated from the supernatants according to the methodology of Pisitkun et al. [1] and Gudehithlu et al. [3] using differential centrifugation. Briefly, urine supernatants prepared as above were centrifuged at 17,000 x g for 20 minutes at 4°C to remove large membrane fragments and other cell organelles. The supernatant was then centrifuged at 200,000 x g (Optima TLX Ultracentrifuge, Beckman Coulter, Inc., Brea CA) for 1 hour at 4°C. Sediment containing exosomes was obtained by decantation of the supernatant. Decantation was carried out by inverting the tubes over a paper towel for 15 minutes to

completely drain the supernatant. Exosomes were solubilized in 500 µL (100 µL in case of rat urine) of PBS containing 0.01% Triton X-100 (PBS-T) (Sigma-Aldridge). Protein concentration was measured in the extracts using Bio-Rad reagent (Bio-Rad Laboratories, Hercules CA). Extracts were adjusted to 1 mg/mL in protein using PBS as the diluent.

Enzyme immunoassay for CP

Ceruloplasmin, levels were quantified in the exosomal extracts using sandwich ELISA kits specific for either human or rat CP (Abcam Inc., Cambridge, MA, USA).

Measurement of urine protein and creatinine

Urinary protein was determined at several dilutions by the Bio-Rad reagent (Bio-Rad Laboratories). Urine creatinine was measured (to 'correct' urine protein levels) at 1:10 and 1:20 dilutions by modified Jaffé reaction using the kit supplied by Sigma-Aldrich.

Histology and histochemistry

For routine histology human biopsy tissues and rat kidney tissues were fixed in 4% formalin and embedded in paraffin. Sections (4–6 µM thick) were obtained and stained with either hematoxylin-eosin (human biopsy) or trichrome (rat kidney) stains. Rat tissues were also collected for cryo-sectioning (without fixing) by applying generous amounts of cryogenic embedding compound (OCT®, Fisher Scientific Inc., Lombard IL) on fresh kidney tissues and then snap freezing them in dry-ice pre-cooled isopentane.

For histochemistry sections were first deparaffinized and treated with citrate buffer (50 mM, pH=6) at 100°C for 10 minutes for re-naturing antigens (also called antigen retrieval). Histochemistry on cryo-sections was performed without deparaffinization and antigen retrieval steps.

Human biopsy tissues were stained for CP by immunoperoxidase technique. But for the primary antibody, all other reagents were supplied by Vector Laboratories, Burlingame, CA, USA. Sections were sequentially incubated with the primary anti-human CP antibody (Abcam Inc., Cambridge MA (catalogue #ab 48614) at 4°C overnight, biotin tagged appropriate secondary antibody (1 hour at room temperature), streptavidin tagged peroxidase (30 minutes at room temperature), and finally with the ABC color regent. All washing steps between incubations were carried out by three 10-minute changes in PBS-T. Sections were cover-slipped with the Vector ® mountant. A human biopsy sample negative for kidney disease was included in the study as a negative control. Antibody controls consisted of sections of each tissue stained identically but for the omission of the primary antibody step. All slides were examined and digitally photographed using an optical microscope (Nikon, New York, NY, USA).

Rat kidney sections were stained for rabbit IgG deposits by direct immunofluorescence technique. Sections were incubated with fluorescene isothiocynate (FITC) tagged anti-rabbit IgG (Sigma-Aldrich) and after washing with PBS-T mounted in 1:1 PBS: glycerol and examined under an epifluorescent microscope and digitally photographed (Nikon).

Rat cryo-sections were stained for CP by indirect immunofluorescence technique by sequentially washing sections with PBS-T, incubating with goat anti-rat CP antibody (Immunology Consultants Laboratory, Inc., Portland OR (catalogue #.GC-25 A-Z)) at 4°C overnight, and finally with FITC tagged anti-goat IgG antibody (Sigma-Aldrich) (1 hour at room temperature). Antibody control consisted of sections of each tissue stained identically but for the omission of the primary antibody step. Slides were mounted in 1:1 PBS: glycerol and examined under an epifluorescent microscope and digitally photographed as above.

Statistical analyses

The data were expressed as mean ± standard error of mean (SEM). Comparisons between groups were tested using one-way ANOVA followed by Tukey's post-hoc test for determining statistical significance among various groups, p values <0.05 were considered significant.

Results

Urine exosomal CP and its localization in the biopsy tissues of non-diabetic CKD patient groups

In order to investigate whether CP is a general marker of kidney diseases we measured CP levels in exosomal extracts of urine from four non-diabetic biopsy-proven CKD (MN, FSGS, lupus nephritis and IgA nephropathy) and a non-biopsy proven CKD stage 1/2 patient groups.

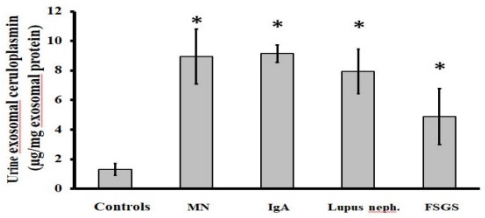

Figure 1: Urine exosomal CP levels in controls and CKD patients of various etiologies. Urine exosomal CP levels in the four biopsy-proven CKD patient groups (membranous nephropathy; MN (N=9), IgA nephropathy (N=7), lupus nephritis (N=10), focal segmental glomerulosclerosis; FSGS (N=10)) were several fold higher than controls (N=12), indicating that high urinary exosomal CP may serve as a general marker of chronic kidney disease of many different etiologies. Similar results were obtained when CP levels were expressed alternatively on basis of 'per mg urinary creatinine' (to 'adjust' CP levels for urine dilution) (not shown). * p<0.05 compared to control.

We found that urine exosomal CP level in the four biopsy-proven CKD patient groups (expressed as 'per mg exosomal protein') was several fold higher than the controls (**Figure 1**),

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showing that high urine exosomal CP appeared to be a general marker of chronic kidney disease of different etiologies. Similar results were obtained when CP level was expressed alternatively on basis of 'per mg urinary creatinine' (to 'adjust' CP levels for urine dilution).

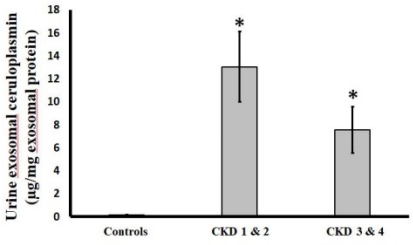

Figure 2: Comparison of urine exosomal CP levels between early and late CKD patients. Urine exosomal CP level in both groups (CKD 1/2 N=28; CKD 3/4 N=24) were several fold higher compared to controls (N=12). Further, urine exosomal CP level in CKD 1/2 group was 1.5 fold higher than CKD 3/4 group (although not statistical significant), indicating that urine exosomal CP level rises early in the course of CKD. * $p<0.05$ compared to control.

To determine whether urine exosomal CP increased early or late in the course of CKD we compared urine exosomal CP levels between CKD 1/2 and CKD 3/4 groups re-sorted from all study patients (**Table 1**). We found that urine exosomal CP level in both groups were several fold higher compared to controls (**Figure 2**). Further, urine exosomal CP level in CKD 1/2 group was 1.5 fold higher than CKD 3/4 group (although not statistical significant), indicating that urine exosomal CP level rises early in the course of CKD.

To validate the biochemically measured high CP in urine exosomal extracts we histochemically stained kidney tissues obtained from biopsy-proven CKD patients for CP. While the control kidney tissue was negative for CP, we found remarkably high CP reactivity present in tubules and collecting ducts (and occasionally in glomeruli) of the kidney tissues from all four non-diabetic biopsy-proven patient groups (**Figure 3**), consistent with the biochemical data shown in (**Figure 1**).

Of note, we observed a poor correlation of urine exosomal CP level with proteinuria in the study patients (R2=0.06; data not shown), further emphasizing that CP in urine exosomes originated from the tubules (as seen by histochemistry) rather than from the proteins filtered from blood.

Histopathology of rat PHN kidney

Histochemically, rats injected with anti-gp600 antiserum showed typical intra-membranous IgG deposits by week 1 which persisted at least until week 3 (**Figure 4**) indicating that PHN was induced soon after injection of the antiserum.

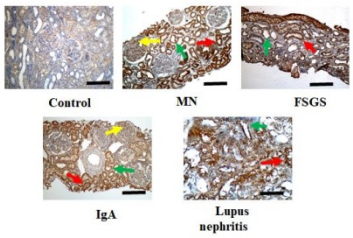

Figure 3: Histochemical detection of CP on kidneys of biopsy-proven CKD patients. Pictures are representative depiction of results from at least three biopsy tissues available from each patient group for immune-staining. While the control human tissue was negative for CP, remarkably high CP reactivity was present in tubules (reactivity is indicated by brown deposits; red arrow) and collecting ducts (green arrows) of kidneys from all patient groups. Reactivity was occasionally also seen in glomeruli (yellow arrows). These results validated the biochemical data of high exosomal CP in urine of these patients shown in **Figure 1**. Note that the picture of lupus nephritis tissue is the best obtained, because for reasons unknown, tissue sections got repeatedly damaged from the antigen retrieval step of immune-staining. The lupus nephritis results, although photogenically wanting, were clearly discernable as presented. Staining: immunoperoxidase. Black bars at bottom of pictures indicate 100 µM length.

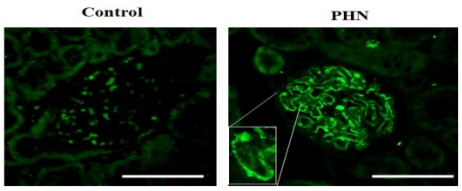

Figure 4: Histochemical detection of rabbit anti-gp600 antibody on PHN rat kidney one week after injection. Passive Heymann nephritis was induced in rats by injection of anti-gp600 antibody. After one week, while the control rat tissue showed only background staining for anti-gp600 antibody, PHN kidney showed high antibody reactivity at the glomerular basement membrane in a granular pattern characteristic of membranous nephropathy. Inset shows the granular staining at higher magnification. Staining: direct immunofluorescence. White bars at bottom of pictures indicate 100 µM length.

While PHN was induced by week 1, no structural changes could be discerned by week 1 and 2 compared to control. However, by week 3 post-PHN extensive loss of brush border in proximal tubules and dilation of tubules were observed (**Figure 5**).

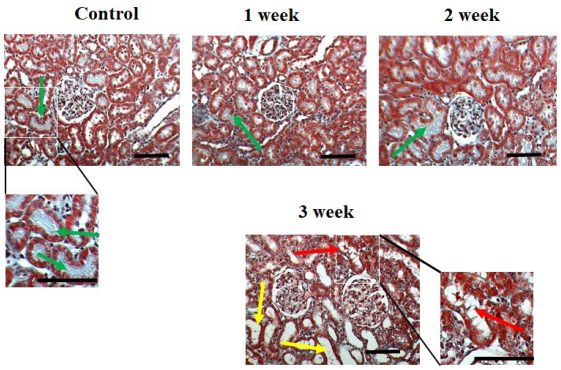

Figure 5: Histopathology of rat PHN kidney. After induction of PHN no structural changes could be discerned by week 1 and 2 compared to control (normal tubular brush border stained blue is indicated by green arrows). However, by week 3 we observed extensive loss of brush border in proximal tubules (indicated by red arrows) and dilation of tubules (yellow arrows). Enlarged areas of control and 3-week photomicrographs show the loss of brush border more appreciably at 3-week time point. Stain: trichrome. Black bars at bottom of pictures indicate 100 µM length.

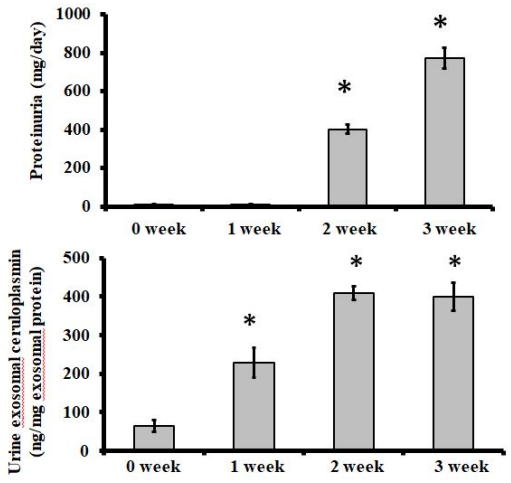

Figure 6: Proteinuria and urine exosomal CP levels after induction of PHN.
Upper panel: After induction of PHN, rats were non-proteinuric (<20 mg/day) until week 1, but presented with proteinuria (>400 mg/day) at week 2 and 3 corresponding to the severe structural changes seen by histology in **Figure 5**. Lower panel: Urine exosomal CP levels increased by 3.5 fold at week 1, and 6 fold by week 2 and 3 (compared to 0 week (control)) after induction of PHN. Comparing the two panels showed that urine exosomal CP increased significantly one week prior to the onset of proteinuria.

Proteinuria after induction of PHN

After induction of PHN, rats were non-proteinuric (<20 mg/day) until week 1, but presented with proteinuria (>400 mg/day) at week 2 and 3 (**Figure 6** upper panel), corresponding to the severe structural changes seen at week 3 by histology (**Figure 5**).

Urine exosomal CP and its localization in the kidney tissue after induction of PHN

Urine exosomal CP levels increased by 3.5 fold at week 1, and 6 fold by week 2 and 3 (compared to 0 week (control)) after induction of PHN (**Figure 6** lower panel). Comparing **Figure 6** upper panel with **Figure 6** lower panel showed that urine exosomal CP increased significantly one week prior to the onset of proteinuria.

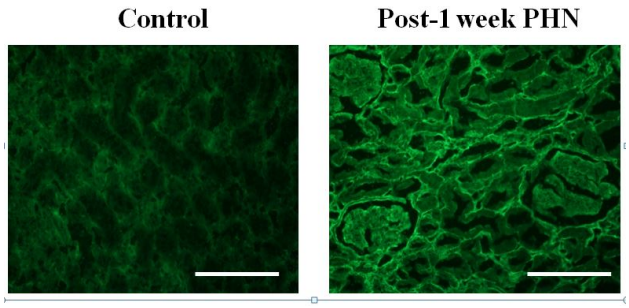

Figure 7: Histochemical detection of CP in rat kidney after induction of PHN. Enhanced CP was detected in the interstitial area of proximal tubule of PHN rats (compared to control) at week 1. Please note that while human kidney CP localized to mostly tubules (**Figure 3**), rat kidney CP (shown in this figure) was found in the interstitial area of the kidney, indicating a species-specific distribution of CP in human and rat kidney. Staining: indirect immunofluorescence. White bars at bottom of pictures indicate 100 µM length.

Increased urine exosomal CP at week 1 was confirmed by enhanced immunofluorescent staining for CP observed in the interstitial area of proximal tubule of PHN rats (compared to control) at week 1 (**Figure 7**). Please note that while human kidney CP localized to the tubules (**Figure 3**), rat kidney CP was found in the interstitial area of the kidney, indicating a species-specific distribution of kidney CP in humans and rats (compare **Figure 3** with **Figure 7**).

Discussion

Previously we had shown that urine exosomal CP level increased in microalbuminuric diabetic kidney disease, and also in a small group of pre-microalbuminuric diabetic patients (3), suggesting that it could be an early marker of diabetic kidney disease. The present study is a follow-up investigation to test whether urine exosomal CP could as well serve as a general marker of underlying kidney disease of other etiologies. To test our hypothesis we first measured urine exosomal CP in four groups of biopsy-proven non-diabetic CKD patients (MN, FSGS, lupus nephritis and IgA nephropathy) and another group of CKD

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1/2 patients (non-biopsy-proven). After we found that urine exosomal CP increased by several fold in all five groups of patients we immune-stained biopsy tissues for CP to identify the source of CP in urine exosomes. In all biopsied cases we found CP to be strongly localized to tubules in the kidney, suggesting that kidney was the source of CP found in urine exosomes. Subsequently, a longitudinal study in rat PHN, a model of human membranous nephropathy, showed that high urine exosomal CP preceded proteinuria in this model, indicating that urine exosomal CP could potentially be an early marker of kidney disease-one that precedes proteinuria.

Upregulation of CP in the kidney tissue has been found to be associated with several kidney diseases, suggesting that it may be a general marker of kidney disease. For example, increased expression of kidney CP has been demonstrated in uranium-induced nephrotoxicity [6,7], cisplatin-induced nephrotoxicity [8], uremic complications of CKD [9], and other toxic insults to the kidney [10]. In addition, increase in urine exosomal CP has been reported in IgA nephropathy and thin basement membrane nephropathy [11]. However, two questions remained to be answered before urinary CP could be considered a clinically useful marker to predict early kidney disease, 1) which compartment (unfractionated urine or urine exosomes) best reflects the changes in kidney CP? and 2) is it a marker that precedes proteinuria?

A definitive answer to the first question, which urinary compartment best mirrors changes in the kidney, was provided by our earlier report which measured two markers, CP and gelatinase, in unfractionated urine and urine exosomes in diabetic nephropathy [3]. We found that underlying changes in the kidney tissue were best reflected in urine exosomes and not in unfractionated urine. The second question, whether increased urine exosomal CP precedes proteinuria, is answered by our present study wherein we demonstrate in a rat model of membranous nephropathy (PHN) that increase in urine exosomal CP occurs prior to proteinuria. Taken together our prior and present studies we are persuaded to believe that urine exosomal CP is a potential early marker of kidney disease of various etiologies, and that it needs to be investigated in human kidney diseases for clinical validation. As a start we are currently evaluating urine exosomal CP as an early marker of diabetic kidney disease (one that precedes microalbuminuria) in human patients.

Is it rational that urine exosomes should reflect changes in the underlying kidney? Exosomes are discrete particles of 10-100 in meters in size which are released by all mammalian cells. It is believed that all mammalian cells release these particles to communicate with each other. Because of the manner by which exosomes are formed and exocytosed from cells it is understood that they would also contain a 'pinch' of cytoplasmic content of the cells of origin. In addition, they contain membrane and nucleic acid (DNA, RNA) signatures of the 'mother' cells they come from [1-2]. Because urine exosomes are released from every segment of the nephron and remain uncontaminated by blood borne exosomes they offer a rich source of kidney disease markers [2]. Unfractionated urine on the other hand mostly contains blood derived substances and only trace amounts of kidney derived metabolites, making their detection difficult.

While it is conceptually reasonable that changes in proteins and other metabolites in the kidney tissue would result in parallel changes in urinary exosomes, it has never been experimentally shown to be so. For example, changes in kidney CP (as by immune-staining) in the studies described above [6-10] did not investigate urinary exosomes to establish this notion. Similarly, authors of reports showing increases of urine exosomal CP in IgA nephropathy and thin basement membrane nephropathy [11] did not immune-stain the kidney tissue to determine whether urine exosomal CP was derived from the kidney. Earlier we reported in diabetic kidney disease that CP expression increased in the kidney and this was confirmed by similar changes in urine exosomal CP [3]. In our present study, by measuring both urinary exosomal CP and immune-staining the kidney (in several types of kidney diseases), we have now established more convincingly that in kidney disease CP is upregulated in the kidney and that its changes are faithfully reflected in urine exosomes.

Is there a physiological basis for an early increase of kidney (and urine exosomal) CP in kidney diseases? Ceruloplasmin is one of the few well-recognized positive acute phase proteins which increase systemically during inflammation or stress. It is a α-2 glycoprotein with an oxido-reductase activity. In addition to being a copper carrying protein, it is also involved in the transport and cellular uptake of iron in the body. This function is performed *via* the oxidation of ferrous ions (Fe^{++}) to ferric ions (Fe^{+++}) which then become competent to be bound and carried by transferrin for the synthesis of hemoglobin. Its oxidase activity inhibits ferrous ion-stimulated lipid peroxidation, prevents formation of hydroxyl radicals in the Fenton reaction, and acts as a scavenger of reactive oxygen species (ROS) [12-14]. While CP is generally considered a blood borne protein, whose source is the liver, it has been speculated that many tissues synthesize their own CP. In support of the kidney being one of such organs, increased expression of kidney CP has been demonstrated in kidney pathologies of various types mentioned above [6-10]. The most-well-studied of the tissue-specific CPs is the brain associated protein which is structurally similar to the circulating CP but contains an additional plasma membrane binding motif, glycosylphosphatidyinositol (GPI) [15,16]. The critical role of the organ specific CP in protecting organ damage is highlighted by the condition of aceruloplasminemia wherein many tissues show tissue damage from excessive iron deposition [13,14]. In that regard our finding of enhanced kidney-derived CP (as also in urinary exosomes) in several kidney diseases suggests that it may be an early protective response against metabolic insults to the kidney, a finding expected for an acute phase protein synthesized in the kidney.

In summary, we first found that urine exosomal CP increased several fold in CKD patients of varied etiologies, unrelated to the degree of proteinuria. In all cases increased urine exosomal CP was strongly associated with enhanced CP in the kidney tissue indicating that urine exosomal CP was of kidney origin and not from filtered blood. Subsequently a longitudinal study in rat PHN showed that urine exosomal CP increased before the onset of

proteinuria, qualifying it as a potential urinary biomarker to diagnose early kidney disease of many different etiologies. It is pertinent to point out that our study has been limited to glomerular kidney diseases. While increased urine exosomal CP appears to reflect a metabolic response to stress in the kidney it will be important to investigate this marker in tubular kidney diseases as well in future.

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