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# Comparison of Surface-Enhanced Raman Scattering Properties of Serum and Urine for the Detection of Chronic Kidney Disease in Patients

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## Abstract

Chronic kidney disease (CKD) affects more than 10% of the global population and is associated with significant morbidity and mortality. In most cases, this disease is developed silently, and it can progress to the end-stage renal failure. Therefore, early detection becomes critical for initiating effective interventions. Routine diagnosis of CKD requires both blood test and urinalyses in a clinical laboratory, which are time-consuming and have low sensitivity and specificity. Surface-enhanced Raman scattering (SERS) is an emerging method for rapidly assessing kidney function or injury. This study was designed to compare the differences between the SERS properties of the serum and urine for easy and simple detection of CKD. Enrolled for this study were 126 CKD patients (Stages 2–5) and 97 healthy individuals. SERS spectra of both the serum and urine samples were acquired using a Raman spectrometer (785 nm excitation). The correlation of chemical parameters of kidney function with the spectra was examined using prinicpal component analysis (PCA) combined with linear discriminant analysis (LDA) and partial least squares (PLS) analysis. Here, we showed that CKD was discriminated from non-CKD controls using PCA–LDA with a sensitivity of 74.6% and a specificity of 93.8% for the serum spectra, and 78.0% and 86.0 % for the urine spectra. The integration area under the receiver operating characteristic curve was $0.937 \pm 0.015$ ($p < 0.0001$) for the serum and $0.886 \pm 0.025$ ($p < 0.0001$) for the urine. The different stages of CKD were separated with the accuracy of 78.0% and 75.4% by the serum and urine spectra, respectively. PLS prediction ($R^2$) of the serum spectra was 0.8540 for the serum urea ($p < 0.001$), 0.8536 for the serum creatinine ($p < 0.001$), 0.7500 for the estimated glomerular filtration rate (eGFR) ($p < 0.001$), whereas the prediction ($R^2$) of urine spectra was 0.7335 for the urine urea ($p < 0.001$), 0.7901 for the urine creatinine ($p < 0.001$), 0.4644 for the eGFR ($p < 0.001$) and 0.6579 for the urine microalbumin ($p < 0.001$). In conclusion, the accuracy of associations between SERS findings of the serum and urine samples with clinical conclusions of CKD diagnosis in this limited number of patients is similar, suggesting that SERS may be used as a rapid and easy-to-use method for early screening of CKD, which however needs further evaluation in a large cohort study.

## Keywords

Surface-enhanced Raman spectroscopy, SERS, chronic kidney disease, renal function, prinicpal component analysis, PCA, linear discriminant analysis, LDA, partial least squares, PLS

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## Introduction

Chronic kidney disease (CKD) is one of major non-communicable disease worldwide, and it affects from 5 to 13% of the general population across different countries.[1] This disease is usually asymptomatic until the later stages, and the diagnosis of CKD relies on blood (creatinine, blood urea) test, serum creatinine (SCr)-based calculation of estimated glomerular filtration rate (eGFR) and sometimes several urinary markers of kidney injury (urinary casts, urinary protein, and fractional excretion of $Na^+$) supplemented with kidney biopsies, which are time-consuming, have poor sensitivity, and specificity.[2,3] Therefore, these methods including surveillance biopsies are impractical for longitudinal disease monitoring.[4,5] Moreover, the eGFR formulae is commonly used to identify patients who are at risk for CKD, but it may either underestimate the eGFR levels of those with normal kidney function[6–8] or overestimate those with CKD or at high risk for CKD.[9,10] Thus, we need new methodology to detect the CKD more specifically and sensitively in this population.

Raman scattering (RS) is a vibrational spectroscopic method that has been used in the chemical analysis by providing fingerprint-type molecular information of a material. This technique has many advantages over the conventional methods for the biochemical testing. For example, it has the capability of simultaneous multiplex detection for one sample, is noninvasive, and requires minimal or no sample preparation.[11,12] RS has also emerged as a qualitative and quantitative assay of real-time molecular profiling in which the spectra can be acquired in seconds with a small sample volume,[13,14] and its applications have been evaluated in many different fields such as food, medicine, and biology.[15–17] However, conventional RS has low sensitivity, a weak signal, and has a strong autofluorescence background that hinders its clinical applications as a diagnostic tool.[11] Surface-enhanced Raman spectroscopy (SERS) is based on the interactions of the target molecules with the surfaces of metal nanoparticles by which the Raman signal of the target molecules can be enhanced up to $10^3$–$10^5$ times.[18,19] Recently, SERS has been used to measure urea, creatinine, and glucose levels in the urine samples of diabetic patients,[20,21] and the urinary SERS spectral features for acute kidney transplant rejection in patients.[22] Additionally, Guo et al.[23] have reported the SERS on human blood samples for CKD detection. Our research group recently showed the use of SERS on urine samples to assess the treatment efficacy in rats with kidney transplants or diabetic kidney disease.[24] All of these studies may suggest that SERS spectral analysis can be used as a convenient method for rapid identification of renal dysfunction in the general population or for monitoring the kidney function of those who are at high risk of developing kidney disease.

In this study, our objective was to use multivariate analyses of correlation of SERS spectra with the kidney functional biomarkers from distinct groups and consequently to verify their feasibility as a potential routine method for rapidly detecting and/or monitoring the kidney conditions including CKD. As compared to the procedure of blood collection, urine collection is relatively simple, convenient, and noninvasive, and we also compared the diagnostic capacity between the serum and urine SERS spectra in order to determine the superiority of the urine SERS as a convenient and noninvasive method for monitoring of kidney health.

## Materials and Methods

### *Patient Cohort*

The Ethics Committee of both Tongji University (Shanghai, China) and the University of British Columbia (Vancouver, British Columbia, Canada) approved this study, and informed consent was obtained from all the subjects for the sample collection prior to the study.

The CKD group comprised 126 patients (73 male and 53 female, Stages 2–5) aged 39–82 years, who visited the outpatient and inpatient department of nephrology from July 2018 to February 2019 at Shanghai East Hospital of Tongji University. These CKD patients were not treated with any renal replacement therapy, and the serum or urine samples from the end-stage (Stage 5) CKD patients that were collected before they received dialysis or transplantation were selected for this study. The CKD with different etiologies was confirmed with both clinical and histopathological diagnoses according to National Institute for Health and Clinical Excellence guideline. After informed consent was obtained, healthy individuals ($n = 97$) were included as the healthy control group, who were admitted purposely for routine health physical examination and were not found any kidney problem when the samples were donated.

All the serum and urine samples for this study were the leftovers otherwise "discarded" after the routine biochemical evaluation of the renal function in a clinical laboratory. The biochemical assay of creatinine and urea was performed by using COBAS E702 Analyzer (Roche Diagnostics Ltd.), whereas the urinary microalbumin (U-MA) levels were determined by using Siemens BNII Chemistry Analyzer (Siemens Diagnostics Ltd.). The patient demographics were listed in Table S1 (Supplemental Material). The clinical stage of CKD was defined according to the eGFR of a patient as follows: Stage 1, indicated by a normal to a high GFR (GFR $>$ 90 mL/min); Stage 2, mild CKD (eGFR: 60–89 mL/min/1.73 $m^2$); Stage 3, moderate CKD (eGFR: 30–59 mL/min/1.73$m^2$); Stage 4, severe CKD (eGFR: 15–29 mL/min/1.73 $m^2$), and Stage 5, end stage of CKD (eGFR: $<$ 15 mL/min/1.73 $m^2$) (Table S2, Supplemental Material).

### *Collection, Processing, and Storage of Both Urine and Serum Samples*

Blood samples were collected from both CKD patients and healthy control subjects after 12 h of overnight fasting. After laboratory test, the remaining serum was kept at −80°C until SERS analysis. There were 223 serum samples (97 control subjects and 126 CKD patients) available for this study. While the remaining urine samples (87 control subjects and 100 CKD patients) were collected after laboratory urinalysis and were stored in aliquots at −80°C until use.

### *Silver Nanoparticles and Sample Preparations for Surface-Enhanced Raman Scattering*

Silver nanoparticles (Ag NPs) were prepared by using hydroxylamine hydrochloride and Ag nitrate as described previously.[24,25] The size of the Ag NPs was confirmed to have a diameter of $35 \pm 5$ nm by transmission electron microscopy as shown in our previous work.[24] Prior to SERS measurement, all Ag NPs and test samples (both urine and serum samples after thawing) were centrifuged using a benchtop centrifuge; the Ag NPs at 10 000 r/min for 10 min, the urine at 1500 r/min for 5 min, and the serum at 5000 r/min for 10 min. The resultant Ag NP pellets and the urine or serum samples were mixed at a 1:1 ratio. The mixture droplets were placed on a rectangle aluminum plate and were dried at 25 °C for 60 min in order to facilitate aggregation of the Ag NPs with the target substances in the samples. If the "coffee ring effect" or crystals formation appeared in the process of the sample Ag NPs preparation, the sample Ag NPs was prepared again until the absence of the crystal effect on the SERS measurement.

### *Surface-Enhanced Raman Scattering Measurements*

The SERS spectra of both the serum and urine with Ag NPs were acquired with a Raman spectrometer (Aura, Verisante Technology) that was equipped with a 785 nm diode laser for Raman excitation as described previously by our group.[26,27] Each SERS spectrum was obtained with 150 mW excitation laser power and one second of an integration time, and with a spectral resolution of 8 $cm^{-1}$ in the wavelength range of 500–1800 $cm^{-1}$. For each sample measurement, three spectra that spotted the whole sample area on the aluminum slide were measured, and the mean of these three spectra was used in the subsequent data analysis. At the outset, the repeatability of the measurements was determined by measuring one spot for 10 times (technical replicates), and the coefficient of variation was <5%.

### *Data Analysis*

The raw signals of SERS spectrum had fluorescence background that were removed using a fifth-order polynomial fitting algorithm prior to data analysis as described before.[28] The differences of the full SERS spectra between groups were examined, and the classification was performed by using prinicpal component analysis (PCA) followed by linear discriminant analysis (PCA–LDA) with the leave-one-out cross-validation (LOOCV) procedure using SPSS software (v.22, SPSS Inc.). Partial least squares (PLS) regression was used to analyze the correlation of the full spectra of SERS with the corresponding kidney function parameters, including creatinine, urea, and MA, and eGFR by using TIBCO Statistica software (v.10, TIBCO Software Inc.). The statistical analysis of the differences between the groups was also performed by using SPSS software. A $p$-value $\leq 0.05$ was considered statistically significant.

## Results

### *Different Surface-Enhanced Raman Scattering Spectra of Serum and Urine Between Chronic Kidney Disease and Control Groups*

As shown in Fig. 1a, the primary peaks of serum SERS spectra in both CKD and control groups were mainly seen at 641, 724, 811, 890, 1003, 1094, 1132, 1210, 1290, 1328, 1398, 1450, and 1655 $cm^{-1}$. The SERS peaks at approximately 1094, 1290, and 1398 $cm^{-1}$ were exclusively presented in CKD patients, while no special prominent peak was seen in healthy controls. The intensity of SERS peaks at 724, 1328, and 1450 $cm^{-1}$ was higher in the CKD patients than that in the healthy controls, whereas the intensity at 641, 811, 890, 1132, 1210, and 1655 $cm^{-1}$ was stronger in the controls than in the CKD patients. We also looked at the changes of these serum SERS peaks corresponding to each stages of CKD (Figure S1, Supplemental Material). The increased intensity at 724, 1094, 1290, and 1450 $cm^{-1}$ was from low at the Stage 2 of CKD to high at the Stage 4, and then went low again at the Stage 5. A similar trend was also seen in the decreased intensity at 640, 1132, and 1655 $cm^{-1}$. The most intensive peaks in the serum spectrum represented a panel of substances (e.g. hypoxanthine, L-tyrosine, amide I, etc.) based on the published data in literature (Table S3, Supplemental Material).

Figure 1b displayed the differences of SERS urine spectra between CKD and the control. The major differences were found at 640, 708, 724, 1006, 1079, 1137, 1185, 1287, 1343, and 1383 $cm^{-1}$. The intensity of SERS peaks at 640, 724, 1137, and 1343 $cm^{-1}$ were higher in controls than that in CKD, while the increased at 1079, 1185, 1287, and 1383 $cm^{-1}$ were specifically found in CKD patients. The changes of the urine SERS in each stage of CKD were also investigated (Figure S2, Supplemental Material). The specific SERS signals for CKD (at 708, 1079, 1185, 1287, 1383, and 1456 $cm^{-1}$) increased from weak at Stage 2 to

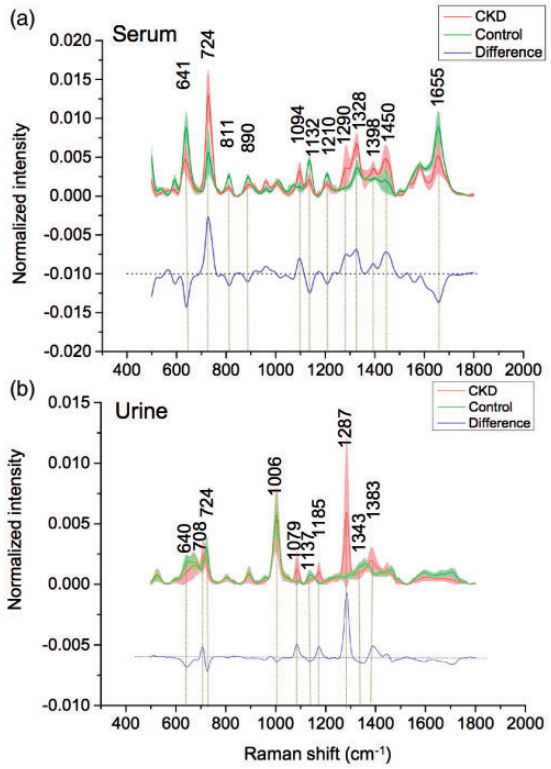


**Figure 1.** Normalized mean and standard deviation (SD) of the SERS spectra of (a) the serum and (b) the urine from healthy control (green line) and patients with chronic kidney disease (CKD) (red line). Shaded area represents the respective SD. Blue line represents the difference between CKD and normal subjects.

strong at Stages 4 or 5. Of further interest, the intensity at 1006 $cm^{-1}$ was higher in CKD at early stages (Stages 2–3) and was lower at late stages (Stages 3–4) than the controls. These marked peaks of urine spectra reflected its major composition (e.g. creatinine, urea, creatine, phosphate, nitrogenous compounds, ketone bodies, and others) based on the published data in literature (Table S4, Supplemental Material).

## Classification Using Principal Component Analysis–Linear Discriminant Analysis

The feasibility of using SERS to differentiate CKD from healthy controls was evaluated by using PCA–LDA analysis with LOOCV. We compared all the PC scores between the healthy controls and the CKD groups using independent sample *t*-test, and found that PC1, PC2, and PC3 using the serum SERS and PC1, PC2, and PC4 using the urine SERS were most diagnostically significant (serum SERS: PC1-3, $p < 0.0001$; urine SERS: PC1 and PC2, $p < 0.0001$, and PC4, $p = 0.0080$) for discriminating the healthy and CKD groups. Figure 2a displayed serum (PC1, PC2, and PC3) and urine (PC1, PC2, and PC4) PC scores from the calculation of the Raman spectra. As observed in the 3D scatter plot in Fig. 2b, the spots of these two groups were separated significantly, indicating that the CKD could be discriminated from the healthy control well. These data suggested that our method of using this LDA discrimination model on the PCs had a high diagnostic ability of the CKD. As shown in Fig. 2c, there was a scattering plot of the linear discriminant scores between the controls and CKD based on the three PCs in Fig. 2a. Using a discrimination threshold of 0, the vast majority of these two groups could be significantly classified. We also used the LOOCV method to validate the LDA discrimination model for 91 of 97 controls (93.81%) and 94 of 126 CKD patients (74.6%), which were classified separately based on the serum spectra; 75 of 87 control subjects (86.2%) and 81 of 100 CKD subjects (81%) were correctly classified with the urine spectra (Table I). The diagnostic sensitivity, specificity, and accuracy for the serum samples were 74.6%, 93.8%, 83.0%, and 78.0%, 86.2%, and 81.8% for the urine samples (Table I). To further assess the PCA–LDA algorithm for classification of the controls and CKD patients, we used receiver operating characteristic (ROC) curves based on posterior probability of each case. In both the serum and urine spectra (Fig. 3a), the posterior probability of the CKD subjects was significantly higher than those of control subjects (both, $p < 0.0001$). Additionally, the area under the ROC curve was $0.937 \pm 0.015$ ($p < 0.0001$) and $0.886 \pm 0.025$ ($p < 0.0001$) for the serum and urine samples, respectively (Fig. 3b).

## Surface-Enhanced Raman Scattering for Discrimination of Chronic Kidney Disease Stages

The application of SERS for identification of CKD stages was further assessed by using the combination of PCA and LDA. We pooled the Stages 2 and 3 (as early stages) and the Stages 4 and 5 (as late stages) for the analysis. As shown in Fig. 4, the majority of the early or late stage cases could be separated from controls based on the SERS spectra of the serum or urine; 95 of 97 control subjects (97.94%), 46 of 81 Stage 2–3 subjects (56.79%), and 33 of 45 Stage 4–5 subjects (73.33%) were cross-validated correctly with the serum spectra, and 70 of 87 control subjects (80.46%), 41 of 64 Stage 2–3 subjects (64.05%), and 30 of 36 Stage 4–5 subjects (83.33%) were identified by cross-validation correctly with the urine spectra (Table II). The diagnostic accuracy in the serum and urine samples was 78.0% and 75.4%, respectively (Table II). The discrimination of each stage of CKD was also performed by using PCA–LDA. As indicated in Figure S3, it was clearly separated between the Stage 3, Stage 4, and control based on the serum spectra, and between the Stage 3, Stages 4 and 5 (combined), and control according to the urine spectra. The correction by using the serum spectra was 95.9% for the control, 100% for the Stage 2, 49.3% for the Stage 3, 60% for the Stage 4, and 60% for the Stage 5, and by using

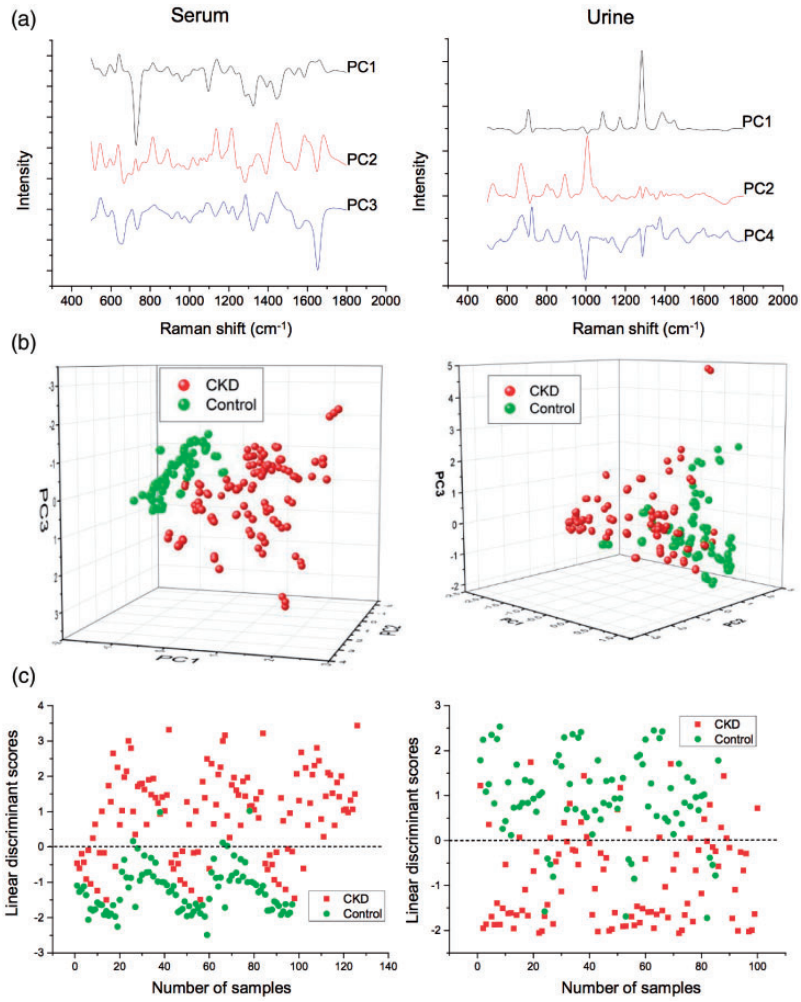


**Figure 2.** Prinicpal component analysis (PCA) of the serum SERS (left) and urine SERS (right). (a) Diagnostically significant principal components for the serum (PC1, PC2, and PC3) and the urine (PC1, PC2, and PC4). (b) 3D scatter plots of the PCA scores for the healthy control and CKD samples from the serum spectra (left) and the urine spectra (right). (c) Scatter plot of LDA scores for the healthy control and CKD samples by using the serum spectra (left) and the urine samples (right).

**Table I.** Classification results of the discriminant analysis of CKD from healthy controls.

| Sample type | Cases | Actual group | Predicted group membership | | Total % of correctly classified cases |
|---|---|---|---|---|---|
| | | | Control | CKD | |
| Serum | Original | Control, *n* (%) | 91 (93.8) | 6 (6.2) | 185 (83.0) |
| | | CKD, *n* (%) | 32 (25.4) | 94 (74.6) | |
| | Cross-validated | Control, *n* (%) | 91 (93.8) | 6 (6.2) | 185 (83.0) |
| | | CKD, *n* (%) | 32 (25.4) | 94 (74.6) | |
| Urine | Original | Control, *n* (%) | 75 (86.2) | 12 (13.8) | 156 (83.4) |
| | | CKD, *n* (%) | 19 (29.0) | 81 (81.0) | |
| | Cross-validated | Control, *n* (%) | 75 (86.2) | 12 (13.8) | 153 (81.8) |
| | | CKD, *n* (%) | 22 (22.0) | 78 (78.0) | |

CKD: chronic kidney disease.

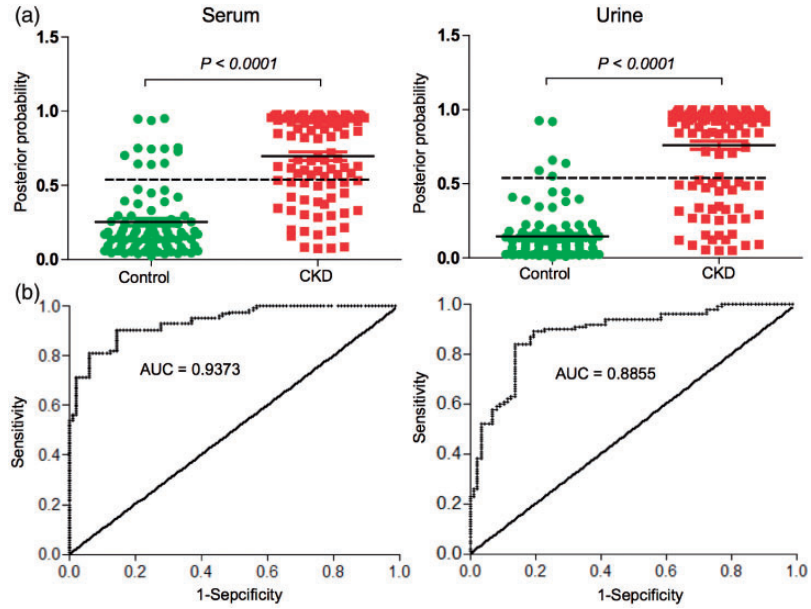


**Figure 3.** The posterior probability of the discrimination results from the serum (left) and the urine (right) spectra utilizing (a) the PCA–LDA spectral classification and (b) the corresponding ROC curves. The area under the ROC curve (AUC) was 0.937 and 0.886, respectively.

the urine spectra was 85.1% for the control, 60% for the Stage 2, 67.8% for the Stage 3, 45.5% for the Stage 4, and 57.1% for the Stage 5 (Table S5, Supplemental Material).

## Correlation of Kidney Functional Parameters of Chronic Kidney Disease Patients with the Serum or Urine Surface-Enhanced Raman Scattering Spectra

The renal function markers, such as creatinine, urea, and others, were superimposed by the protein peak due to its higher concentration in the samples, as noted in Fig. 1, so that a multivariate spectral model based on the PLS regression was built to predict the levels of each kidney function-related biochemical component in both the serum and urine samples. Figure 5a displayed the predicted results of the serum urea, SCr, and eGFR according to the serum spectra. These PLS models with the predicted levels and the levels of those chemicals determined by the routine colorimetric methods were presented, resulting in $R^2 = 0.8540$ for the serum urea ($p < 0.001$), $R^2 = 0.8536$ for the SCr ($p < 0.001$), $R^2 = 0.7500$ for the eGFR ($p < 0.001$). The estimate root mean square error of cross validation (RMSEcv) that was calculated from predicted samples were 2.47 mmol/L for the serum urea, 31.83 mmol/L for the SCr, and 7.68 mL/min/1.73 $m^2$ for the eGFR. The chemical levels predicted by the urine spectra were shown in Fig. 5b. The PLS models presented high correlation coefficients for the urinary urea ($R^2 = 0.7335$, $p < 0.001$) and urinary creatinine (UCr) ($R^2 = 0.7901$, $p < 0.001$), and also U-MA ($R^2 = 0.6579$, $p < 0.001$). The relatively low correlation was found to be $R^2 = 0.4644$ for the eGFR ($p < 0.001$). The RMSEcv from predicted urine samples were 632.44 mmol/L for the urinary urea, 4.19 mmol/L for the UCr, 10.50 mmol/L for the U-MA, and 13.93 mL/min/1.73 $m^2$ for the eGFR.

## Discussion

There are many pathophysiological risk factors for CKD, such as age, obesity, diabetes, and hypertension.[5,29] Thus, a single biomarker cannot fully describe the loss of renal function due to its complex pathophysiology. Therefore, using a panel of multiple biomarkers, rather than a single marker, may be more specific and/or more sensitive in detection of or diagnosis of different stages of CKD. However, detecting the changes of various markers in a large panel requires tedious sample preparation, numerous reagents, long output times, and extensive analyses, which thus increases overall healthcare costs and the time for the detection and diagnosis. Alternatively, SERS, a method of providing a global chemical profile of a sample, emerges as a lucrative option for rapid disease detection with no sample preparation involved, nondestructive and nonintrusive analysis, high sensitivity, and even subtle biochemical perturbations that are less time consuming.[30,31] In our previous studies,[24] SERS was used to explore the assessment of treatment efficacy using the urine samples of rats with kidney transplants or native kidney disease in which we provided evidence of using the SERS to monitor the renal impairment.

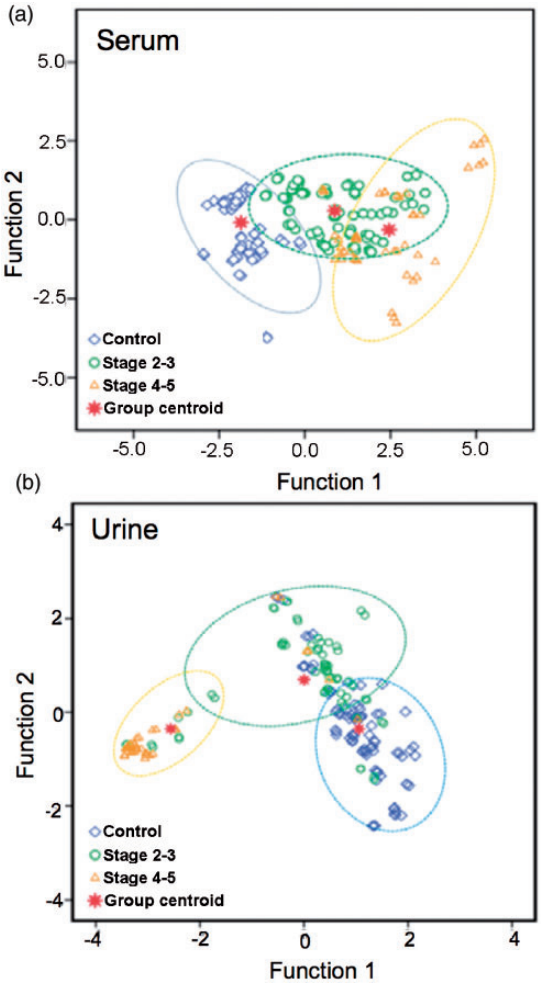


**Figure 4.** Scatter plot of PCA–LDA scores for the stages of the CKD by using the (a) serum spectra and (b) the urine samples. Function 1 or 2 was interpreted as linear discriminant component 1 or 2.

In the present study, we further evaluated the feasibility of using either the serum or urine SERS as a novel tool to detect the CKD from healthy population, and to differentiate the different stages of this disease.

The SERS spectra of the sera have been used for the discrimination of the CKD patients from normal healthy controls.[23] In this study, the primary SERS peaks at 641, 724, 813, 1003, 1132, 1210, 1326, 1450, 1583, and 1655 $cm^{-1}$ are reported in both healthy controls and CKD patients. Similarly, the present study showed the peaks of serum SERS spectra at 641, 724, 811, 1003, 1132, 1210, 1328, 1450, 1584, and 1655 $cm^{-1}$ (Fig. 1a). These signals may be attributed to some known biochemical components, such as L-tyrosine and lactose with C–S vibration structure (641 and 813 $cm^{-1}$), nucleic acids (641, 724, 813, 1003, 1210, and 1450 $cm^{-1}$), carbohydrates (641, 890, and 1094 $cm^{-1}$), lipids (1278 and 1328 $cm^{-1}$), and amino acid (1655 $cm^{-1}$) (Table S3, Supplemental Material).

The Raman spectral feature of human urine has also been reported in two different studies. Stefancu et al.[32] have reported the primary Raman peaks at 680, 1002, 1026, 1204, and 1650 $cm^{-1}$, and Chi et al.[22] have shown peaks at around 675, 772, 1000, 1028, 1125, 1264, 1301, 1350, and 1620 $cm^{-1}$ in which there are four overlapping peaks at 680/675, 1002/1000, 1026/1028, and 1650/1620 $cm^{-1}$. In the present study, we detected three of them (640–680, 1006, and 1625–1655 $cm^{-1}$) (Fig. 1b), The missing peak at 1026/1028 $cm^{-1}$ was probably too close to the strong peak at 1006 $cm^{-1}$. Such other predominant Raman signals as at 1079, 1185, 1287, and 1383 $cm^{-1}$ in the CKD group have not been reported in literature, which may be specific to this group of the CKD population. These peaks may represent nitrogenous compounds (C–N stretching from primary amines) and cytosine (Table S4, Supplemental Material). The nitrogenous compounds, mainly seen as ethanolamine bands at 880 and 1079 $cm^{-1}$,[33,34] are probably generated from the metabolism of amino acids in the urea cycle and may be higher in the urine of CKD patients. Similarly, cytosine is one of the four main bases in both DNA and RNA, and it is derived from metabolites in cellular processes, which are also associated with CKD progression.[35] Furthermore, the renal function can be determined by the levels of the urea, presented by the peaks at 527, 1006, and 1159 $cm^{-1}$, and the creatinine, indicated by the peaks at 605, 681, and 848 $cm^{-1}$ (Fig. 1b), which are similar to the results reported in the previous studies.[36] Although more studies of these spectral changes are needed, it is possible that the differences of these peaks are related to renal pathological changes and metabolism in the CKD patients.

In order to develop the classification and diagnostic models between the CKD and controls, PCA–LDA analysis with LOOCV was used to analyze the SERS spectra as previously reported.[36,37] In the present study, the resultant algorithm could successfully classify and diagnose the CKD from the healthy group with a sensitivity of 74.6%, a specificity of 93.8%, and an accuracy of 83.0% using the serum spectra, or with 78.0%, 86.2%, and 81.8% using the urine spectra, respectively (Table I; Fig. 2). The ROC analysis confirmed the diagnostic algorithms according to either the serum or urine SERS spectra, and the integrated AUCs were 0.94 and 0.89, respectively. These new data further suggest the diagnosis value of the SERS for the kidney disease or transplant rejection as reported previously.[23,24]

However, the discrimination between the stages of CKD by either the serum or urine spectra was not such obvious (Fig. 4 and Figure S3, Supplemental Material), but it was significantly clear between the healthy controls and the CKD patients. One of the reasons could be due to the limited numbers of the samples in each group, particularly of the patients with the Stage 2 ($n = 6$) and the Stage 5 ($n = 15$) (Table S2). Due to the variability of physiological

**Table II.** Classification results of CKD stages from the PCA–LDA model based on the SERS spectra of the serum and urine using the LOOCV method.

| Sample type | Cases | Actual group | Predicted group membership | | | Total % of correctly classified cases |
|---|---|---|---|---|---|---|
| | | | Control | Stage 2–3 | Stage 4–5 | |
| Serum | Original | Control, *n* (%) | 95 (97.9) | 2 (2.1) | 0 (0) | 179 (80.3) |
| | | Stage 2–3, *n* (%) | 9 (11.1) | 49 (60.5) | 23 (28.4) | |
| | | Stage 4–5, *n* (%) | 0 (4.0) | 10 (22.2) | 35 (77.8) | |
| | Cross-validated | Control, *n* (%) | 95 (97.9) | 2 (2.1) | 0 (0) | 174 (78.0) |
| | | Stage 2–3, *n* (%) | 12 (11.1) | 46 (60.5) | 23 (28.4) | |
| | | Stage 4–5, *n* (%) | 0 (4.0) | 12 (22.2) | 33 (77.8) | |
| Urine | Original | Control, *n* (%) | 71 (81.6) | 16 (18.4) | 0 (0.0) | 142 (75.9) |
| | | Stage 2–3, *n* (%) | 13 (20.3) | 41 (64.1) | 10 (15.6) | |
| | | Stage 4–5, *n* (%) | 1 (2.8) | 5 (13.9) | 30 (83.3) | |
| | Cross-validated | Control, *n* (%) | 70 (80.5) | 17 (19.5) | 0 (0.0) | 141 (75.4) |
| | | Stage 2–3, *n* (%) | 13 (20.3) | 41 (64.1) | 10 (15.6) | |
| | | Stage 4–5, *n* (%) | 1 (2.8) | 5 (13.9) | 30 (83.3) | |

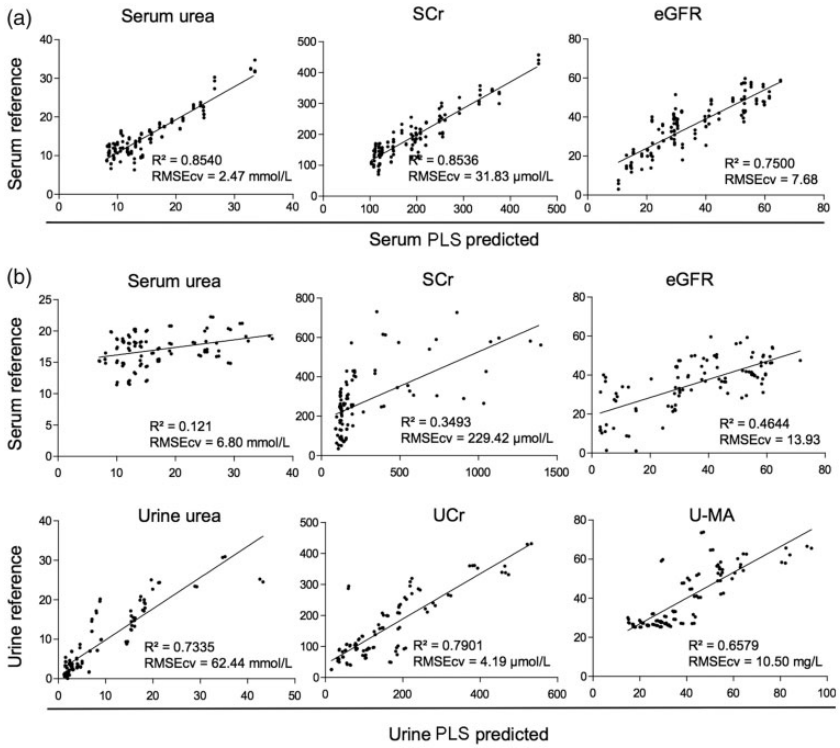


**Figure 5.** Correlation of kidney functional parameters of CKD patients with the (a) serum and the urine SERS spectrum, predicted by PLS versus the levels of the biochemical substance or eGFR measured by the chemical assay. The resulting $R^2$ and the RMSEcv are presented for each parameter of the kidney function.

or metabolic conditions between the humans, these sample sizes may not be big enough to differentiate the stages of CKD. The second reason could be that all the Raman signals were included in the discrimination analysis, and some of them were not linearly related to the CKD stages. For example, after normalization with the healthy controls, the intensity of the Raman signal of the urine spectra at 1006 $cm^{-1}$ was higher in the Stage 2, but it was lower in

the Stages 4 and 5 (Figure S2). Thus, identification of CKD stage-dependent signal(s) or chemical composition with unknown special NPs is required for a reproducible discrimination between the stages of CKD in future studies. Nevertheless, it was found that both the serum and urine spectra had a "good" performance in the analysis and classification of the CKD stages with a total accuracy of 78.0% and 75.4%, which further confirmed a great potential of the SERS for monitoring the development and progression of the CKD (Tables II and S5, Supplemental Material).

Although similar results were found by using either the serum or urine samples in this study, the urine sample is considered to be an attractive source for renal biomarker analysis because it is a product from the kidney, and it can be collected by a noninvasive and convenient manner. When the diagnostic capabilities of the SERS were compared between the serum and urine specimens, we also found that the sensitivity of using the urine spectra was equally effective or might be a little better than that of the serum (74.6% for the serum and 78.0% for the urine), implying that the urine SERS may be preferred in the application for the screening and monitoring of the renal conditions.

Our SERS data also indicated a strong relationship of the serum SERS spectra with the levels of the serum urea, creatinine, and eGFR (Fig. 5). Although these markers have little reliability,[38] the urine spectra presented the high correlation coefficients for the urine urea and creatinine, and as well for U-MA (Fig. 5b), despite of the lower correlation for the both serum urea and SCr (Fig. 5b). The values of RMSEcv and correlation coefficients in this study are similar to the previous studies in literature. For example, Wang et al. have used the SERS to measure the creatinine levels in both artificial and human urine samples. They report a correlation coefficient of $r = 0.99$ in the artificial urine samples over the range of 3.3946–13.6139 mmol/L, and $r = 0.96$ in the human urine samples of 0.2263–10.1662 mmol/L.[39] Similarly, based on the Raman urea and creatinine bands, and their levels in the urine measured by the colorimetric method, PLS model resulted in $r = 0.90$ and 0.91 for the urea and creatinine with a RMSEcv of 312 and 25.2 mg/dL, respectively.[40] Taken together, our study and these studies in literature might demonstrate that the SERS is a promising technique for the qualification of some urine and serum biomarkers in the response to the development and progression of CKD.

One has to acknowledge the limitations of the present study as follows: (i) there was a technical issue by using Ag NPs to enhance the Raman signaling that often formed a coffee ring in the sample preparation due to the poor stability of these colloidal NPs. Thus, finding new nanomaterials with specific properties for Raman spectroscopy analysis of the serum or urine specimens is required for further development of this technology in this field; (ii) this is a preliminary study of this project with a small number of patients. Further studies with a large, multicenter cohort in a blinded fashion are needed to confirm the Raman spectroscopy as a simple, rapid diagnostic tool for the CKD.

## Conclusion

The SERS approach is a feasible tool for identifying the differences in the biochemical constituents of the serum and urine samples between the CKD and healthy control subjects. By using the samples from patients with different stages of CKD, we showed the feasibility of the SERS as a routine, rapid, and simple method to assess the kidney conditions. Our data suggest that the SERS analysis of either the serum or urine samples combined with the PCA–LDA method has promising potential for rapid assessment of the CKD stages. In consideration of the inconvenient procedure of blood collection, the urine samples, collected by a noninvasive method, may be preferred in the SERS rapid screening of the CKD in the general population.

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### Supplemental material

All supplemental material mentioned in the text, consisting of three figures and five tables, is available in the online version of the journal.

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