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Development of a method for optical monitoring of creatinine in the spent dialysate

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Abstract. The aim of the study was to develop a method suitable for the estimation of the amount of creatinine, removed during dialysis through UV-absorbance. Sixteen uremic patients, seven females and nine males, on chronic thrice-weekly hemodialysis were included in the study. Double-beam spectrophotometer was used for the determination of UV-absorbance in the collected spent dialysate samples. Due to differences in independent variables, two multi-wavelength models (m1 and m2) were developed using stepwise regression, utilizing creatinine values from the laboratory as the dependent parameter. The coefficient of determination, R^2 was 0.8690 for the first and 0.9103 for the second model. The systematic error, estimated as *BIAS*, was zero for both models compared to the creatinine values from the laboratory. The standard errors were 10.06 μmol/l and 15.24 μmol/l for m1 and m2, respectively. The average reduction ratio (*RR*) from creatinine blood values was 59.8 ± 5.4% ($N = 50$), average *RR* from m1 was 63.7 ± 7.3% ($N = 50$) and average *RR* from m2 was 64.8 ± 6.4% ($N = 48$). In summary, the amount of creatinine removed as well as the reduction ratio of creatinine during dialysis can be estimated with UV-absorbance technique.

Key words: hemodialysis monitoring, creatinine, UV-absorption.

1. INTRODUCTON

One of the substances, which are retained in uremic patients and are eliminated from blood by hemodialysis, is creatinine. It is a breakdown product of creatine phosphate in muscle cells and the concentration in blood depends primarily on the muscle mass. Creatinine is usually produced at a constant rate. It is removed from

the plasma by glomerular filtration and then excreted in the urine without any appreciable reabsorption by the tubules. Typically from 7% to 10% of creatinine in the urine is derived from tubular secretion but this is increased in the presence of renal insufficiency. Because creatinine is endogenous and is freely filtered at the glomerulus, it is widely used to assess kidney function (Glomerular Filtration Rate, GFR) and is expressed either as a plasma concentration or renal clearance. Elevated levels of plasma creatinine are associated with impaired renal function. Increase in serum creatinine is the result of uremic retention, but can also be due to muscle breakdown [1]. A higher level of serum creatinine has been proved to be an independent significant predictor of long-time survival in incident dialysis patients [2]. Creatinine is considered as a non-toxic reference molecule for the removal of water-soluble low-molecular-weight uremic retention solutes whose kinetic behaviour may differ from that of urea [3,4].

Creatinine-based parameters, creatinine index (*CI*) and lean body mass (*LBM*), have been suggested as excellent predictors of long-term survival in dialysis patients [5]. It has also been shown that *LBM* has an association with increased relative risk for having cardiovascular disease [6]. One of the advantages of creatinine-based indices is that they reflect somatic protein metabolism [5]. Low creatinine outputs as well as reduction in creatinine indexes during HD therapy serve as strong predictors of muscle dystrophy [7]. It has been suggested that small muscle mass is an important risk factor for patient mortality in HD [8].

One of the widely accepted ways to determine creatinine is the Jaffe method, which utilizes picric acid for the Jaffe Reaction to test for creatinine [9]. It forms a coloured bright orange-red complex that can be measured using spectroscopy. The enzymatic colorimetric determination of creatinine largely eliminates interferences known in the Jaffe method. The demerits of the abovementioned methods are the following: 1) some compounds similar to creatinine, contained in the sample of biological fluid, may affect the test accuracy, 2) the operation is complex, needs lots of agents, which are hard to keep and should be operated by professionals, 3) the sample must be de-protein pre-treated and 4) the necessary equipment is expensive.

Thus there is a need for a new method, which can directly and easily perform quantitative concentration measurements of water-soluble small molecular weight substance creatinine in biological fluid (e.g. in spent dialysate) and avoids the disadvantages, caused by the analysis in the laboratory. This would make estimating the parameter *LBM* a suitable automatic and time-efficient way to review the muscle mass and protein nutritional status of HD patients. An optical method, utilizing UV-absorbance, has been proposed for the monitoring of dialysis adequacy [10–12]. An earlier study has indicated that the multiwavelength UV-absorbance method may improve the measurement accuracy for optical creatinine concentration estimation in spent dialysate in terms of relative error, compared to the algorithm, based on the single wavelength approach [13]. This study was undertaken to explore the effect of complementary parameters related to solute transport in the dialyser and patient.

The aim of the study was to develop a method, suitable for the estimation of creatinine concentration, removed during dialysis through UV-absorbance.

2. PATIENTS

After approval of the protocol by the Regional Ethics Committee, Linköping, Sweden and by Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia, a total of 16 patients on chronic thrice-weekly hemodialysis were studied in two separate studies at the Department of Nephrology, University Hospital, Linköping (study 1) and at the Department of Dialysis and Nephrology, North-Estonian Medical Centre, Estonia (study 2). An informed consent was obtained from all participating patients.

Treatment times ranged from 240 to 300 minutes and the dialysate flow was fixed at 500 ml/min.

Study 1. Six uremic patients, four females and two males, were followed during a total of 44 hemodialysis sessions. Two different polysulphone dialysers, F50 ($N = 20$) and F6 HPS ($N = 24$) (Fresenius Medical Care, Germany), with the effective membrane area of 1.0 and 1.3 m^2, respectively, were used. The blood flow varied between 160 and 350 ml/min. The type of dialysis machine used was AK200 (Gambro Lundia AB, Sweden).

Study 2. Ten uremic patients, three females and seven males, were followed during a total of 30 hemodialysis sessions. Three different polysulphone dialysers, F8 HPS ($N = 14$), F10 ($N = 3$), and FX80 ($N = 13$) (Fresenius Medical Care, Germany), with effective membrane areas of 1.8, 2.2 and 1.8 m^2, respectively, were used. The blood flow varied between 245 and 350 ml/min. The type of dialysis machine used was Fresenius 4008H (Fresenius Medical Care, Germany).

3. MATERIALS AND METHODS

For both studies, samples of blood and spent dialysate were taken at discrete times for the analysis (Table 1). The numbers for "Sampling time" correspond to minutes after the start of hemodialysis. Blood and dialysate samples were taken at 270 and 300 min when the duration of the session was long enough. The concentration of creatinine was determined using standardized methods. The accuracy of the methods for the determination of creatinine in dialysate and blood was ±5%.

Double-beam spectrophotometer (in Linköping UVIKON 943, Kontron, Italy; in Tallinn Shimatsu UV-2401 PC, Japan) was used for the determination of UV-absorbance in the collected spent dialysate samples. Spectrophotometric analysis over a wavelength range of 190–380 nm was performed by an optical cuvette with an optical path length of 1 cm. Some of the measured values (absorbance or concentration) were excluded from data before analysis. The exclusion criteria

Table 1. Discrete sampling of blood and spent dialysate

Study	Sampling time, min	
	Dialysate	Blood
1	2, 4, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300	0, 4, 10, 20, 30, 60, 120, 180, 240, 270, 300
2	10, 60, 120, 180, 240	0, 240

were incorrect or illogical values of the measured concentration or absorption, e.g., sampling coexisting with self-tests of the dialysis machine.

Stepwise regression, i.e., forward stepwise, with creatinine concentration in spent dialysate as the dependent variable, was used to obtain a multiwavelength model for the assessment of creatinine concentration through UV-absorbance. In order to build up an optimal model, two data sets were developed including different independent variables in the analysis.

Model 1. Independent variables included were UV-absorbance values at wavelengths 210–330 nm.

Model 2. Independent variables included were: 1) UV-absorbance values at wavelengths 210–330 nm, 2) urea clearance of the dialyser, 3) time, when the sample was taken, 4) the patient's weight after dialysis, 5) systolic blood pressure after dialysis, 6) diastolic blood pressure after dialysis and 7) a dummy variable "Diabetes", which had a value of "1" if the patient was diabetic and a value of "0" if the patient was not.

The wavelength range around 290–330 nm has been proposed as suitable for instrumental design because the highest correlation coefficient r value for uric acid, creatinine and urea was obtained at wavelengths from 280 to 320 nm [14]. On the other hand, the highest absorbance sensitivity for substances like creatinine, uric acid and phosphate was obtained in the wavelength range of 220–270 nm [14].

Systematic error $BIAS$ was calculated for the two models as follows:

$$BIAS = \frac{\sum_{i=1}^{N} e_i}{N}, \tag{1}$$

where e_i is the residual and N is the number of observations.

Standard error SE was calculated for the two models as follows:

$$SE = \sqrt{\frac{\sum_{i=1}^{N} (e_i - BIAS)^2}{N-1}}. \tag{2}$$

The reduction ratio of creatinine was calculated from the concentration at the start of hemodialysis (C_{start}) and concentration at the end of hemodialysis (C_{end}) as follows:

$$RR = \frac{C_{\text{start}} - C_{\text{end}}}{C_{\text{start}}} 100\%. \quad (3)$$

The value of RR was calculated based on creatinine concentration in blood (RRb) as well as creatinine concentration in dialysate predicted by model 1 (RRm_1) and model 2 (RRm_2). In case of blood, the creatinine concentration from the sample, drawn before the start of the treatment, was utilized as C_{start}. In case of creatinine concentration in dialysate, predicted by models 1 and 2, the value 10 min after the start of hemodialysis was utilized as C_{start}. Student's t-test was used to compare means of RRb, RRm_1 and RRm_2, and $p \le 0.05$ was considered significant. The RRm_1 and RRm_2 values were compared with RRb values also using Bland and Altman analysis [15].

For the analysis, Statistica 6.0 (Statsoft, Inc. for Windows) and Excel (version 2000 for Windows) were used.

4. RESULTS

The analysis of the first data set resulted in a model (model 1), including the wavelengths 218, 303 and 326 nm. Figure 1 shows the scatter plot of creatinine concentration estimated by model 1.

The analysis of the second data set resulted in a model (model 2), including the wavelengths 214, 307, 326 nm and the parameters diabetes as a dummy variable, weight after dialysis, systolic blood pressure after dialysis and diastolic blood pressure after dialysis. Figure 2 shows the scatter plot of creatinine concentration estimated by model 2.

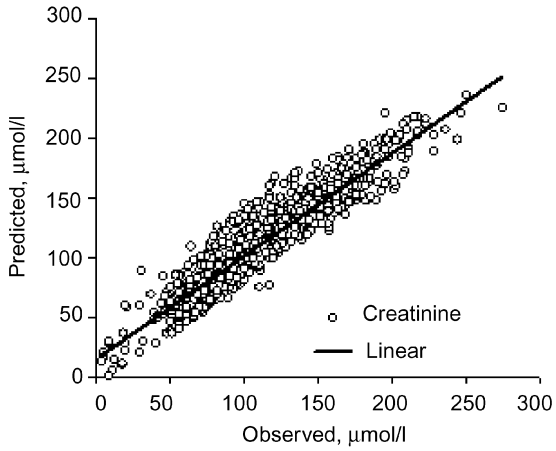

Fig. 1. Scatter plot of the creatinine concentration in dialysate, determined at the laboratory vs predicted by model 1.

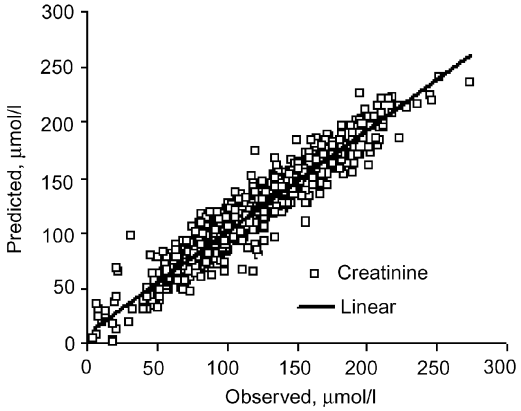

Fig. 2. Scatter plot of the creatinine concentration in dialysate, determined at the laboratory vs predicted by model 2.

The correlation coefficients, coefficients of determination and number of cases of the two models are presented in Table 2. Figure 3 shows the dependence of the residuals on the creatinine concentration, determined at the laboratory for model 1, and Fig. 4 the same for model 2. The systematic error, estimated as

Table 2. The correlation coefficients, coefficients of determination and numbers of cases of the two models

Model	Multiple R	Multiple R^2	N
1	0.9322	0.8690	721
2	0.9541	0.9103	705

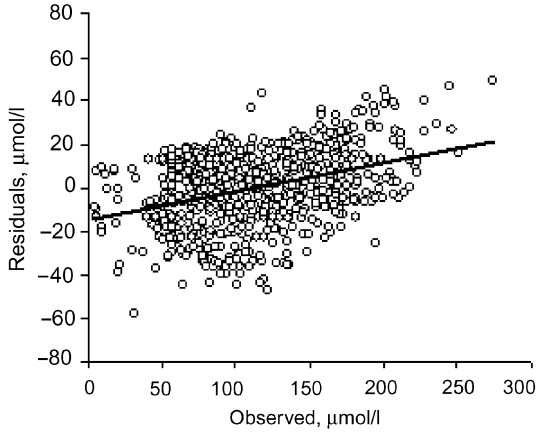

Fig. 3. Scatter plot of creatinine concentration in dialysate, determined at the laboratory vs residuals for model 1.

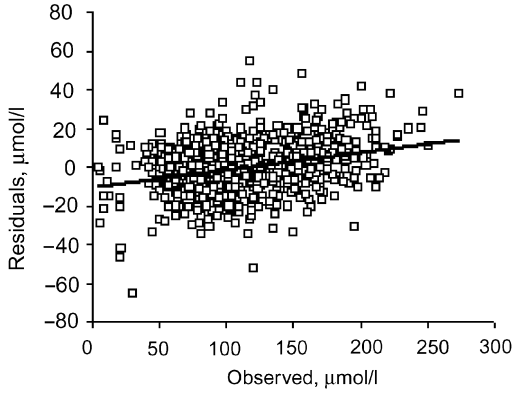

Fig. 4. Scatter plot of creatinine concentration in dialysate, determined at the laboratory vs residuals for model 2.

BIAS, was zero for both models. The standard error of the model 1 was 10.06 µmol/l and 15.24 µmol/l for model 2.

The average *RRb* of creatinine was 59.8±5.4% ($N = 50$), average RRm_1 was 63.7±7.3% ($N = 50$) and average RRm_2 was 64.8±6.4% ($N = 48$). The average RRm_1 and RRm_2 values were slightly higher compared to the *RRb* value ($p = 2.1\times10^{-5}$ and $p = 1.7\times10^{-5}$, respectively). Figure 5 shows the Bland–Altman plot of the differences between *RRb* and RRm_1. The mean difference between *RRb* and RRm_1 was −3.8±5.6%. Figure 6 shows the Bland–Altman plot of the differences between *RRb* and RRm_2. The mean difference between *RRb* and RRm_2 was −5.0±7.0%.

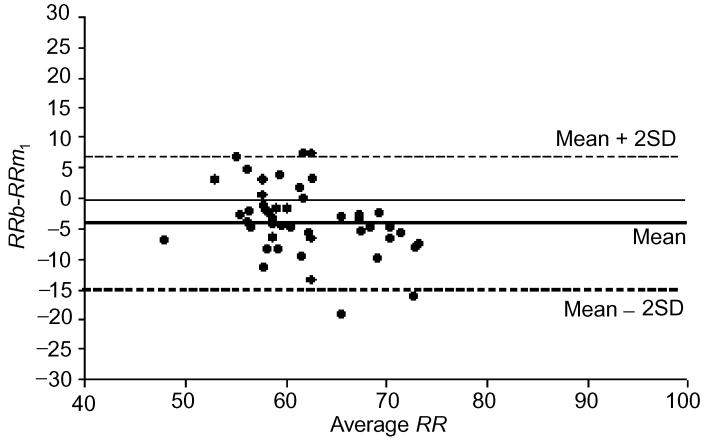

Fig. 5. Bland–Altman plot of the differences between *RRb* and RRm_1.

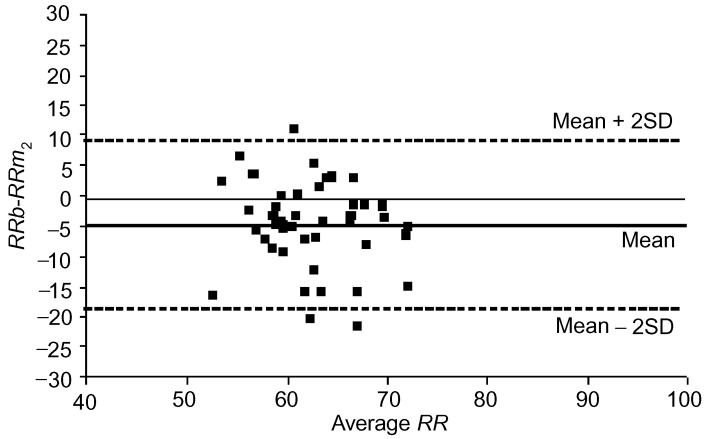

Fig. 6. Bland–Altman plot of the differences between RRb and RRm_2.

5. DISCUSSION

Regression analysis resulted in two models, utilizing several wavelengths and parameters. A higher coefficient of determination was obtained for model 2 (Table 2). On the other hand, the standard error of the estimate was higher for model 2.

The millimolar extinction coefficients for creatinine have two distinct maxima around 202 and 235 nm and one minimum around 220 nm [14]. Model 1 included the wavelength 218 nm and model 2 the wavelength 214 nm. Generally, the wavelengths at which the absorbance for the particular chromophores is characteristic, such as regions of flat maxima and minima, are preferable. The presence of UV-absorbance at the wavelength 303 nm in model 1, UV-absorbance at the wavelength 307 nm in model 2 and UV-absorbance at the wavelength 326 nm in both models is in accordance with the fact that the highest correlation coefficient r value for creatinine has been found to be at wavelengths from 280 to 320 nm [14].

The fact that model 2 includes a patient-dependent parameter like weight after dialysis indicates that the relationship between creatinine and UV-absorbance is related to the patients' muscle mass, where creatinine is actually produced. This influence seems to be more complex than can be predicted by stepwise regression analysis assuming a linear relationship between independent and dependent parameters. Moreover, the diabetic status of the patients also plays a significant role in model 2, probably pointing out a specific metabolic behaviour affecting the correlation between UV-absorbance and creatinine concentration in spent dialysate.

It appears that in the future, model 2 cannot be implemented unless the dialysis session is finished and the patient weighed. To overcome this impediment, the patient's weight after the previous dialysis session could be used for monitoring the creatinine in spent dialysate during dialysis.

The value of RR was slightly overestimated when calculated from the creatinine concentration in dialysate, predicted by the models 1 and 2 compared to RR, obtained utilizing blood creatinine. This difference could be explained by specific removal characteristics for creatinine compared to the net removal of the UV-absorbing chromophores contributing to the optically measured signal in spent dialysate. Despite that the online UV-absorbance at 280 nm was closest to the removal of small water-soluble non-protein bound solutes like urea, creatinine and uric acid [16,17], the overall removal pattern is far more complex because each uremic solute has still a distinctive distribution volume in the body and removal rate during dialysis. This is expressed also by independent components in the models, including several wavelengths, proposed in this study. The differences between RRb and RRm_1 are smaller (Fig. 5) than the differences between RRb and RRm_2 (Fig. 6) in accordance to the results from the regression analysis for the creatinine concentration measurements. However, the study demonstrates that UV-absorbance at the wavelengths, included into the models, gives a good estimate to the removal pattern of the small water soluble non-protein bound solute creatinine during dialysis. This makes it possible to monitor creatinine even when the technique does not measure solely creatinine but several UV chromophores in the spent dialysate.

In summary, although the second data set included more variables, the model (model 2) that was revealed by analysis did not give significantly better results than model 1. The standard error was higher for model 2. Also, RRb and RRm_2 show less agreement than RRb and RRm_1. Consequently, model 1 seems preferable to model 2. To validate the algorithm with experimental material, not included into the model build-up, and to explore if the proposed method in this paper could be applied for distinguishing patients with different diets (e.g. low vs high protein diet) will be an issue of further studies. Also, estimation of the total removed creatinine could be useful for the assessment of dialysis quality and protein nutritional status of the patients.

6. CONCLUSION

The results show that it is possible to estimate the amount of creatinine, removed during dialysis, using UV-absorbance. Additional parameters as diabetes, weight and blood pressure appear to have an impact on the estimation. The merits of the described method are that it does not need blood samples and disposables of chemicals and is fast.

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Meetodi väljatöötamine kreatiniini optiliseks seireks dialüsaadivedelikus

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Kreatiniin on väikeste vees lahustuvate ureemiliste jääkainete klassi kuuluv lihasrakkude ainevahetuse lõppsaadus, mis eemaldatakse organismist dialüüsravi käigus. Artiklis on näidatud, et dialüüsi käigus eemaldatud kreatiniini kontsentratsiooni on võimalik hinnata, kasutades UV-sumbuvust dialüsaadis. Parameetrid diabeet, kaal ja vererõhk avaldasid kreatiniini kontsentratsiooni hindamise täpsusele täiendavat mõju. Kirjeldatud optilise meetodi eeliseks on, et mõõtmisi on võimalik teha reaalajas ja meetod ei vaja vereproove ega kemikaale ning on kiire.