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A multicentre study of an enhanced optical method for measuring concentration of uric acid removed during dialysis

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Abstract— **The aim of this study was to compare concentration measurements of uric acid (UA) removed during dialysis by two algorithms based on UV-absorbance and the 1st derivate of UV absorbance.**

Ten uremic patients from Tallinn and ten from Linköping, during 30+40 haemodialysis treatments, were followed at the Departments of Dialysis and Nephrology at North-Estonian Medical Centre and at Linköping University Hospital. The dialysate samples were taken and analyzed by means of UA concentration at the chemical laboratory and with a double-beam spectrophotometer. UV absorbance and derivate of UV absorbance was transformed into UA concentration in the spent dialysate using the regression models from the calibration set of material, noted as UV-absorbance (UV_A) and the 1st derivate of UV absorbance (UV_D) method. These models were tested on validation set of material and concentrations of UA from the two methods were compared regarding mean values and SD.

Mean concentration of UA were 52.7 ± 25.0 micromol/l measured at the chemical laboratory (UA_Lab), 54.9 ± 23.8 micromol/l determined by UV_A and 52.9 ± 23.0 micromol/l determined by UV_D. The results of mean concentrations were not significantly different ($p \geq 0.54$). The systematic errors were -7.8 % and -3.3% and random errors were 15.8 % and 10.4 % using UV_A and UV_D respectively. The systematic and random errors were significantly different ($p < 0.05$) indicating that the new algorithm enables more accurate UA estimation.

I. Introduction

MONITORING of the important biological constituents in spent dialysate can prevent serious pathological conditions and decrease mortality of patients. There is a need for a simple, compact, inexpensive, mobile and reliable method for measuring concentration of uremic toxins in the spent dialysate.

Uric acid, a final product of the metabolism of purine (MW=168,1) is mostly excreted from human body through the kidneys in the form of urine. The concentration of uric acid in blood increases when the source of uric acid increases or the kidney malfunctions. High level of serum UA, hyperuricaemia, has been suggested to be an independent risk factor for cardiovascular and renal disease especially in patient with in heart failure, hypertension and/or diabetes [1]-[5]. Also is hyperuricaemia a novel risk factor for type 2 diabetes mellitus [6] and has also shown to be the cause of renal disease in a rat model [7].

UA is removed from plasma in a similar manner as urea during dialysis treatment [8] but so far has not been investigated concerning patient outcome, compared to urea. UA is mostly associated with gout, but studies have found that UA affects biological systems [9], [10] and could also cause higher mortality in dialysis patients [10]. According to European Society of Cardiology guidelines 2008 for the diagnosis and treatment of heart failure (HF) elevated UA level is associated with a poor diagnosis in HF and UA is one of the biomarker in HF [11].

A good correlation between ultra-violet (UV)-absorbance in dialysate and the concentration of several solutes both in the spent dialysate and in the blood of the dialysis patients has been shown in earlier studies, indicating that the technique can be used to estimate the removal of retained substances [12]. The possibility to estimate dialysis dose (urea-*Kt/V*) [13] and total removed urea by UV-absorbance [14] has been presented. Also it is shown that the removed UA can be reliably estimated in different dialysis centres by the UV technique [15].

The aim of this study was to compare concentration measurements of UA removed during dialysis by two algorithms based on UV-absorbance and the 1st derivate of UV absorbance using data from two different dialysis centres.

II. Materials and Methods

This study was performed after approval of the protocol by the Regional Ethics Committee, Linköping, Sweden and by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. An

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informed consent was obtained from all participating patients.

Ten uremic patients, three females and seven males, mean age 62.6 ± 18.6 years, were included in the study at the Department of Dialysis and Nephrology, North-Estonian Medical Centre, Estonia and 10 uremic patients, four females and six males, mean age 62.8 ± 20.9 years were included at the Department of Nephrology, University Hospital of Linköping, Sweden, using the clinical set-up of the experiments as described earlier [15]. All patients were on chronic thrice-weekly haemodialysis. The patients were monitored during three dialysis treatments in Tallinn and four dialysis treatments in Linköping with duration from 240 to 270 minutes (totally 70 haemodialysis sessions). The studied treatments were consecutive in Tallinn and not consecutive in Linköping but were performed within three weeks for each patient.

In Linköping an althane dialyser was used with the effective membrane area of 1.8 m2 (AF180, Ahltin Medical, Ronneby, Sweden). The dialysate flow was 500 mL/min and the blood flow was 300 mL/min except in one session (250 mL/min) due to temporary access (needle) problems. Two types of machines were used, AK 200 (Gambro Lundia AB, Sweden) and Fresenius 4008H (Fresenius Medical Care, Germany).

In Tallinn F8 HPS (N=14), F10 (N=3), and FX80 (N=13) (Fresenius Medical Care, Germany) with the effective membrane area of 1.8 m2, 2.2 m2, and 1.8 m2 were used, respectively. The dialysate flow was 500 mL/min and the blood flow varied between 245 to 350 mL/min. The type of dialysis machine used was Fresenius 4008H (Fresenius Medical Care, Germany).

Dialysate samples were taken in the beginning and 5 (only in Linköping OIL), 10 (only in Tallinn), 15 (OIL), 30 (OIL), 60, 90 (OIL), 120, 180 minutes after the start of the dialysis session and at the end of the session (210, 240 or 270 minutes). Also sample from the total dialysate collection, marked as "tank" was included into analysis in Tallinn.

Pure dialysate was collected before the start of a dialysis session, used as the reference solution, when the dialysis machine was prepared for starting and the conductivity was stable. The concentration of UA was determined at the Clinical Chemistry Laboratory at North-Estonian Medical Centre and at the Clinical Chemistry Laboratory at Linköping University Hospital using standardized methods.
Double-beam spectrophotometers (UVIKON 943, Kontron, Italy) in Linköping and (SHIMATSU UV-2401 PC, Japan) in Tallinn were used for the determination of UV-absorbance. Spectrophotometric analysis over a wavelength range of 190 - 380 nm was performed by an optical cuvette with an optical path length of 1 cm. (Fig. 1a).
The obtained UV-spectra's were processed with a signal processing tool using Savitzky-Golay algorithm for smoothing and the first derivative calculation. (Fig. 1b). The data acquisition module consisted of a PC incorporated in the spectrophotometer using UV-PC software (UV-PC personal spectrophotometer software, version 3.9 for Windows). The obtained UV-absorbance values were processed and presented by software Panorama fluorescence and the final data processing was performed in EXCEL (Microsoft Office Excel 2003).
Data of 15 patients was used to generate models for estimating concentration of UA (calibration set). For that regression line of the collected dialysate samples and corresponding UV-absorbance values at the wavelength 298 nm and derivative spectra values at the wavelength 301 nm was assessed to transform UV-absorbance into UA concentration. The obtained relationships were used on the rest 5 patient's data (validation set) for calculating concentration of UA, comparing those values with laboratorial results and estimating different models.
For a single session Accuracy was in percentage as Accuracy = 100*(UA_Lab–UA_A)/UA_Lab, when calculated for a UV_A method. UA_D was used instead of UA_A when accuracy was calculated for a UV_D method. Random error was calculated for different methods as SD over the sessions' accuracy. Student's t-test (two tailed) and f-test was used to compare means and accuracy values for different methods.

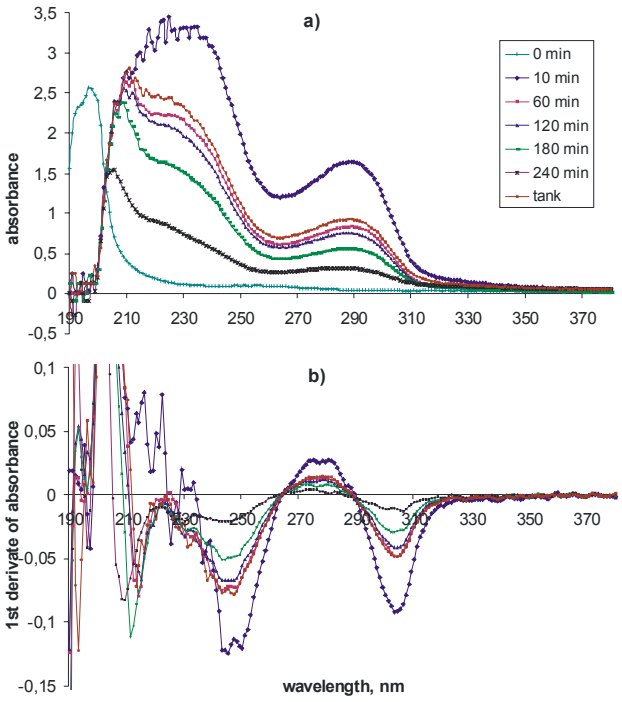

Fig. 1 An example of the absorbance spectrum (a) and 1st derivate of absorbance spectrum (b) obtained over a wavelength range 190-380 nm on the spent dialysate samples at different times during a dialysis session

III. Results

On the basis of the laboratorial UA concentration, measured UV absorbance spectra and processed UV absorbance, spectra linear correlation coefficients for UA was determined. (Fig. 2).

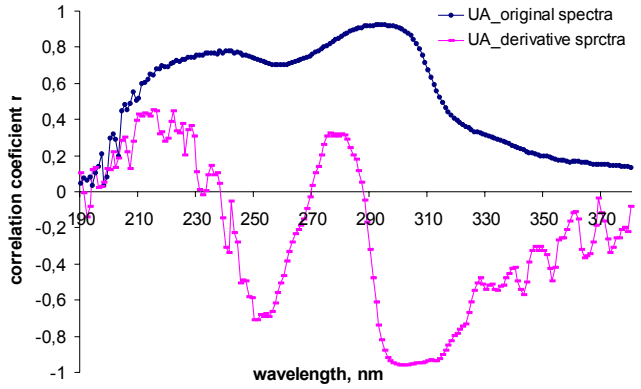

Fig. 2 Value of correlation coefficient r between original and derivate of UV absorbance over a wavelength range of 190-380 nm and concentration of UA in the spent dialysate

Figure 3 shows the linear relationship between UA concentration and UV absorbance at 298 nm in the case of calibration set.

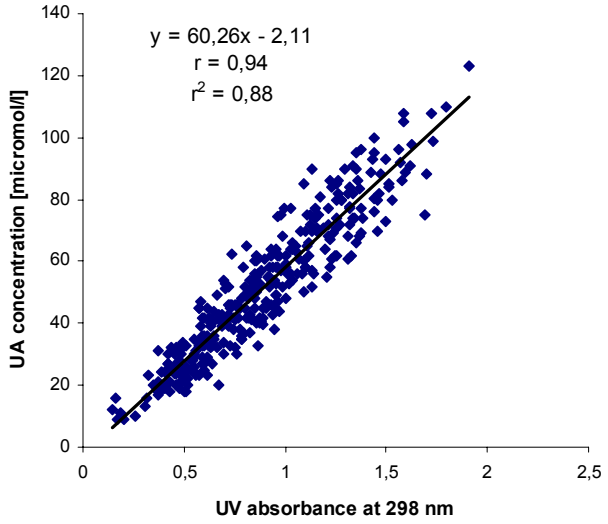

Fig. 3 A regression line between UA concentration in dialysate and UV-absorbance at 298 nm (r = 0.94, N = 335)

Figure 4 shows the linear relationship between UA concentration and 1st derivate of UV absorbance at 301 nm in the case of calibration set.

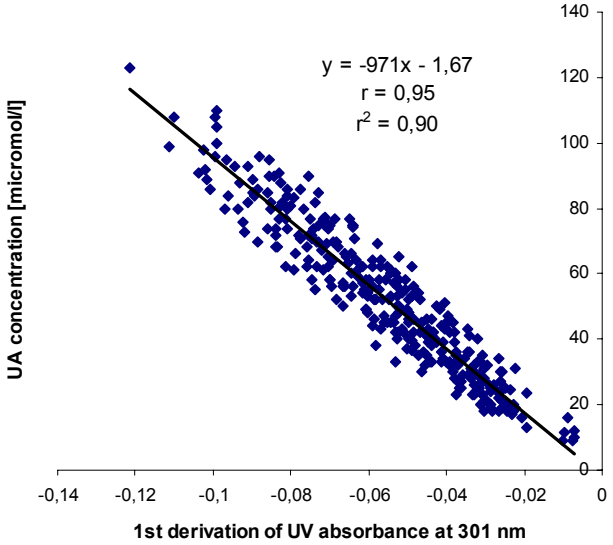

Fig. 4 A regression line between UA concentration in dialysate and 1st derivate of UV-absorbance at 301 nm (r = -0.95, N = 335)

The relationships used for generating concentration calculation algorithms to estimate UA concentration were y = 60.26x – 2.11 for original UV-absorbance spectra and y = -971x – 1.67 for the derivative spectra. These models were applied on validation set of data (25% from the total material – 5 new patients) and concentrations of UA were calculated.

Table 1 shows the mean values of the concentrations and errors of the UA measured at the laboratory and UA calculated from two different models for the validation set.

TABLE I
SUMMARY OF RESULTS FOR THE DIFFERENT METHODS TO MEASURE CONCENTRATION OF THE URIC ACID

	UA_Lab	UA UV_A	UA UV_D
N	135	135	135
Concentration of UA ± SD (micromol/l]	52.66 ± 25.1	54.87 ± 23.8	52.91 ± 23.0
Syst. Error [%]	-	-7.81 %	-3.26 %
Random Error [%]	-	15.8	10.4

These results demonstrate that using a processed UV absorbance for calculating concentration of UA, more reliable results will be achieved.

Both, systematic and random errors were significantly different ($p < 0.05$) indicating that the 1st derivate algorithm enables more accurate UA estimation.

These results shows that utilizing the UV_D the concentration of the UA can be predicted more reliably and accurately in terms of systematic and random error.

IV. Discussion

The presented results show the possibility to estimate UA concentrations with two models based on UV-absorbance and the 1st derivate of UV absorbance.

A good possibility to estimate UA concentrations using UV technique has shown in earlier studies [15] but if we use some signal processing tools we can essentially improve the accuracy and reliability of the results.

Linear correlation coefficient r between laboratorial and calculated values of UA is slightly higher in case of the UV_D compared to the UV_A (0.95 vs. 0.94). Table 1 shows that the systematic error is 2 times and random error is 6 % lower in case of UV_D than with the UV_A. Considering improvement in systematic and random error, the signal processing should be certainly used in the future.

The high correlation between UV-absorbance and UA could be explained by the characteristic absorbance around 294 nm for UA in combination with relatively high millimolar extinction coefficients of UA in this wavelength region compared to other chromophores-uremic retention solutes eliminated from blood into the spent dialysate during dialysis [16]. This makes it possible to determine UA concentration even when the technique does not measure solely UA. The use of derivative spectra is a good method for correcting baseline effects in spectra and this could explain the improvement in accuracy.

The clinical aim in the future is to develop an on-line monitoring system that could offer an estimation of the removal of several clinical important solutes during haemodialysis. If there is an accurate determination method, it is possible measure UA rapidly and on-line without need for blood samples and disposables-chemicals. Using quite simple signal processing tool might be very helpful for achieving more accurate and reliable results. The UV technique for measuring concentration of UA may give a useful, rapid and cost-effective tool for clinicians to assess removal of this biomarker as a potential independent risk factor for renal failure and cardiovascular disease.

V. Conclusion

This study investigated the effect of signal processing to an optical method to estimate concentration of UA using UV absorbance. It was found that smoothing and the first derivative of absorbance spectra leads to more accurate results. As UA is shown to be an independent risk marker of cardiovascular and renal disease, and also a novel risk factor for type 2 diabetes mellitus [1]-[6], it is advantageous to develop reliable and rapid methods for uric acid concentration measurements.

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