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Estimation of Removed Uremic Toxin Indoxyl Sulphate during Hemodialysis by Using Optical Data of the Spent Dialysate

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***Abstract*— The aim of this study was to explore the possibility to determine the amount of total removed Indoxyl Sulphate (TR_IS) during dialysis session, an optical method utilizing absorbance and fluorescence spectral data of the spent dialysate was used. Eight uremic patients from Linköping, Sweden and 10 from Tallinn, Estonia, were studied during dialysis treatments. Dialysate samples were taken during each treatment and analyzed at a laboratory. Fluorescence and absorbance spectra of the spent dialysate were measured with spectrofluorophotometer and spectrophotometer. The spectral values were transformed into IS concentration using multiple linear regression model from the total material noted as optical method (Opt). IS concentration was estimated using high-performance liquid chromatography (HPLC) method as a reference. TR_IS values were calculated. Achieved results were compared regarding mean values and SD and collated with the amount of total removed urea value (TR_Urea) for the same dialysis procedures. Mean TR value±SD (mg) for urea was 28 947±9 241; TR for IS was 151.4±87.3 estimated by HPLC and 149.4±84.9 estimated by Opt. The TR_IS values were not significantly different ($p \leq 0.05$). This study indicates, that it is possible to estimate TR_IS using only spectral values of the spent dialysate and the parameter can be used for quantifying the elimination of protein bound uremic toxins during the dialysis procedure.**

I. Introduction

Uremic toxins can be divided in three large groups: small, water-soluble, not protein bound solutes (molecular weight (MW) < 300D), middle molecules (300<MW<12000 D) and protein bound molecules. Indoxyl Sulphate (IS) (MW 251 D) belongs to protein bound uremic toxins. IS is metabolized by the liver from indole which is produced by the intestinal flora as a metabolite of tryptophan [1, 2]. It has found that serum IS level is markedly higher in chronic kidney disease patients and it accelerates the progression of chronic kidney disease (CKD) [3]. Moreover, studies suggest that IS increases oxygen consumption and aggravates local hypoxia in renal tubular cells and can lead to end stage renal disease (ERSD) [4]. It has discovered that IS may induce oxidative stress, dysfunction of endothelium and affect endothelial wound repair, those conditions may cause cardiovascular disease and higher mortality in CKD patients [5-8]. The present concept of dialysis as the main treatment in case of ERSD, focuses mainly on the removal of small water-soluble compounds [2]. Also the classical ways to estimate dialysis adequacy is based on small water-soluble compounds as urea and creatinine [9]. Monitoring the amount of removed protein bound molecules during dialysis is important and informative. However, due to specific kinetic behavior of some protein bound molecules in the body (including IS), the removal estimation on the basis of blood samples could be misleading [10]. Earlier studies by HPLC have shown that IS can be observed by fluorescence measurements in the plasma as well as in ultrafiltrate [11, 12]. It has been demonstrated that concentration and removal rate (RR) of different uremic solutes can be estimated with optical methods which are using either ultraviolet absorbance [13-15] or fluorescence [16-18] of the spent dialysate. The aim of the study was to enhance current knowledge about using spent dialysate fluorescence and absorbance data to quantify total removed IS (TR_IS) during dialysis.

II. Materials and Methods

The studies were performed after approval of the protocol by the Regional Ethical Review Board, Linköping, Sweden and by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. Informed consent was obtained from all participating patients.

10 patients from Tallinn (6 male, 4 female, 58±8 years) and 8 patients from Linköping (7 male, 1 female, 77±7 years) were included to the study. Patients were followed in total 78 sessions from which 33 were hemodialysis (HD) and 45 hemodiafiltration (HDF) sessions. The dialysis machine used was a Fresenius 5008 (Fresenius Medical Care, Germany). The dialyzers used were FX10, FX80 and FX800 (Fresenius Medical Care, Germany). The duration of the treatments varied between 180 to 270 minutes, the dialysate flow was 500 ml/min and the blood flow varied between 250-350 ml/min.

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During dialysis sessions, the following samples from the drain tube of the dialysis machine were taken: 9-25, 60, 120 minutes after the start of the session and at the end of the session (180-270 min.). After the end of the procedure, dialysate collection tank was weighed and one sample (Tank) was taken from it after careful stirring. Linköping's samples were freezed and transported to Tallinn.

Concentration of IS was determined in Tallinn University of Technology utilizing the HPLC instrument UltiMate 3000 (Dionex Corporation). Concentrations of urea were determined in Clinical Chemistry Laboratories in Sweden and Tallinn using standardized methods (ADVIA Urea Nitrogen (UN) method, enzymatic).

Spectrofluorophotometer (SHIMADZU RF-5301, Japan) was used for the fluorescence measurements. Fluorescence analysis was performed over an excitation (EX) wavelength range of 220 - 500 nm (excitation increment 10 nm) and emission (EM) wavelength range of 220-800 nm. The measurement cell with an optical path length of 4 mm was used (Fig. 1).

Ultra violet (UV) absorbance was measured with UV-VIS-NIR spectrophotometers (V-570, JASCO Corp., Japan-used in Sweden and UV-3600, SHIMADZU, Japan – used in Tallinn), 10 mm optical cell was used (Fig. 2). Measurements were performed at the room temperature (ca. 22^{o} C). The obtained spectral values were processed and presented by software Panorama fluorescence and UV Probe, the final data processing was performed in (Microsoft Office Excel 2003).

Linear correlation coefficients (R) were determined on the basis of the fluorescence/absorbance spectral values and IS concentration values using MATLAB (MATLAB 7.0, MathWorks, US) and EXCEL. The best wavelengths for estimating the concentration of IS were found and multiple linear regression model utilizing information from those wavelengths was created using Statistica 9.0 (Statsoft Inc., US). The obtained model was in following form:

$$IS = \sum_{i=0}^{N} a_i * F(\lambda_i) + \sum_{j=0}^{M} b_j * A(\lambda_j) \qquad (1)$$

where a and b are the coefficients, F(λ) is fluorescence intensity value at certain EX/EM wavelength and A(λ) is UV absorbance value at certain wavelength.

The total removed amount of a substance was calculated as follows:

$$TR = C_t * W_t \qquad (2)$$

where C_t is the substance concentration in total dialysate collection tank (mg/l) and W_t is the weight of the dialysate collection tank (kg). It was assumed that 1 kg = 1 liter of the dialysate. For determination of the TR_IS from the optical method (Opt), concentrations calculated from the spectral values were utilized.

For determining differences between TR values from HPLC and optical method Student t-test ($p<0.05$ was considered significant) and Bland-Altman analysis was used.

III. Results

In the Fig. 1 fluorescence spectrum of the spent dialysate in the beginning and at the end of the procedure is given, corresponding absorbance spectra's are shown in Fig. 2.

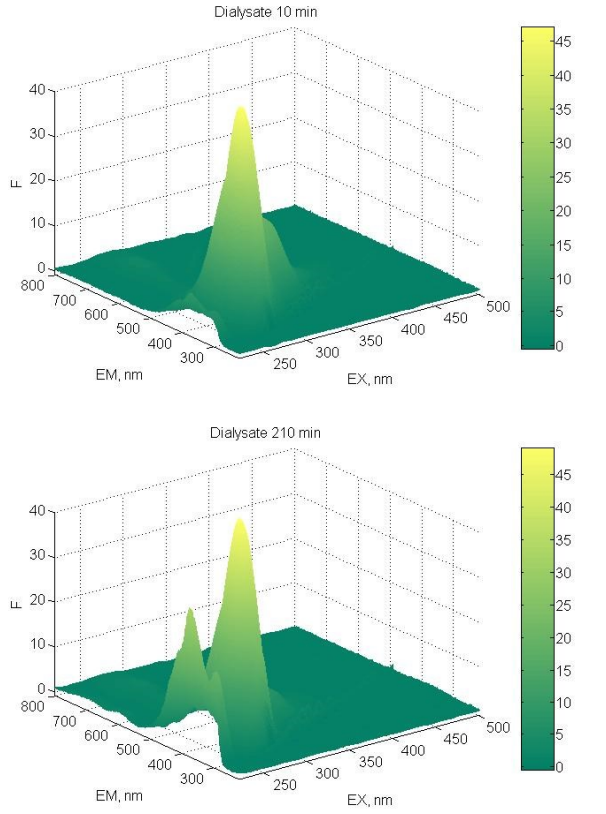

Figure 1. Examples of fluorescence spectra's of spent dialysate, taken at the start and at the end of the dialysis procedure. EX=220-500nm, EM=220-800nm.

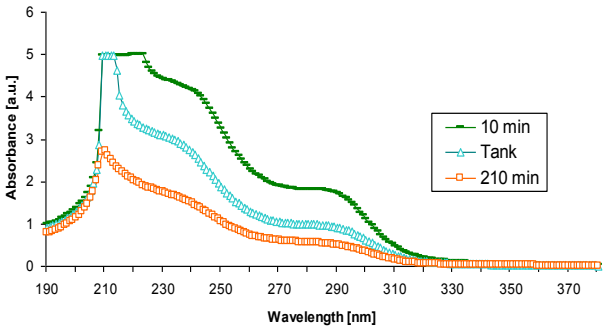

Figure 2. Examples of absorbance spectra's of spent dialysate samples taken at the start and at the end of the dialysis procedure and from dialysate collection tank over a wavelengt range 190-380 nm.

The multiple linear regression model for estimating IS concentration optically (IS_Opt) was created. The goodness of fit of the model is given in the Fig. 3. and mean concentration values of urea and IS estimated with different methods are presented in the Fig. 4.

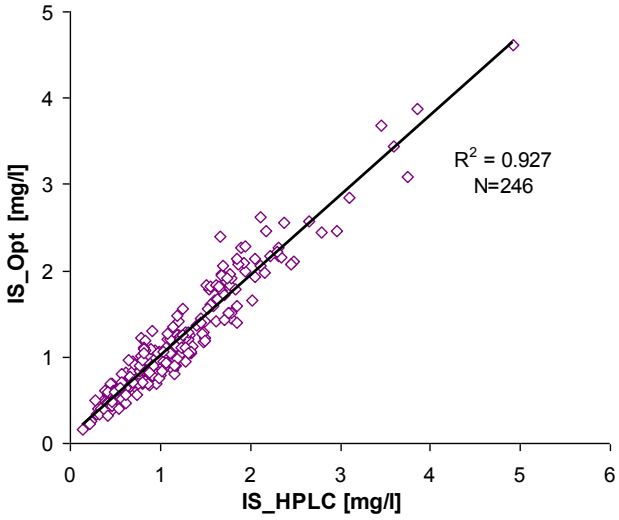

Figure 3. Goodness of fit of the multiple linear regression model for estimating IS concentration.

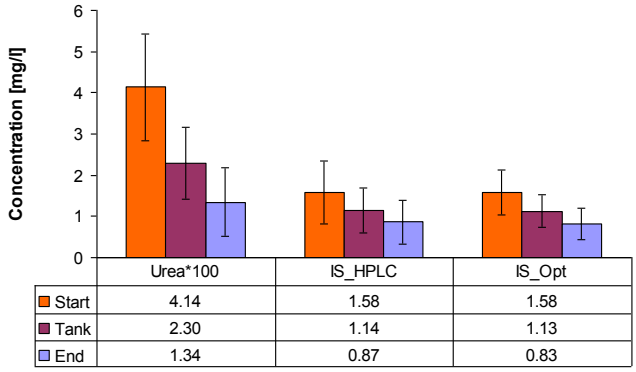

Figure 4. Urea and IS concentration values.

The concentration values from the tank were used for calculating the TR value for urea and IS (both HPLC and Opt). The results are presented in the Fig. 5.

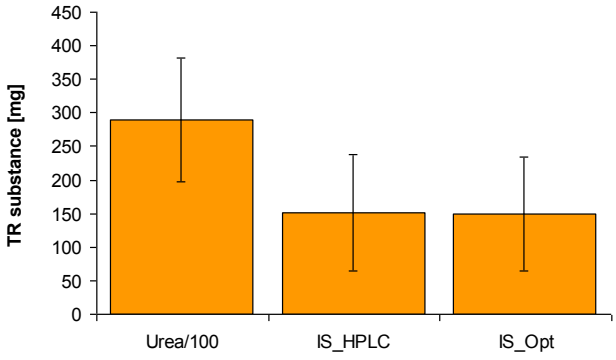

Figure 5. Amount of total removed urea and IS measured with the different methods.

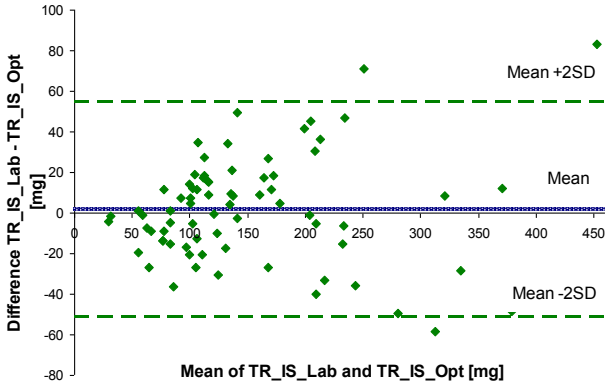

Figure 6. Bland-Altman plot. The difference between TR_IS_HPLC and TR_IS_Opt is plotted against the mean value of TR_IS_HPLC and TR_IS_Opt, (N=74).

Fig. 6 presents the individual differences between TR_IS values estimated by HPLC (reference method) and by the new optical method (Opt), in a Bland-Altman plot.

Neither concentrations nor TR values of IS_Opt were significantly different ($p<0.05$) from the values of the reference method (HPLC).

Table 1 shows a summary of the results regarding the TR values of IS in mean and standard deviation values from the standardized methods (IS_HPLC) and new optical method (IS_Opt), and correlation analysis between two methods.

TABLE I. TOTAL REMOVED IS ESTIMATED BY DIFFERENT METHODS (N=74)

	TR_IS ± SD[mg]	***R***	***R²***
HPLC	151.4±87.3	0.95	0.91
Opt	149.4±84.9		

IV. DISCUSSION

From the Fig. 1 and Fig. 2, some distinctive fluorescence and absorbance maxima at specific regions are clearly seen. The amplitude of the spectra's are proportional to the content of eliminated uremic retention solutes in the spent dialysate being higher/lower in the beginning of the dialysis treatment (10 min) and lower/higher at the end of the dialysis (210 min) at specific regions.

According to the HPLC studies on the heat-deproteinized uremic serum and uremic ultrafiltrate, IS has a prevalent fluorescence compared to other uremic retention solutes [19]. Therefore using fluorescence data in optical model is well justified. It has been confirmed by the HPLC studies of the spent dialysate that UV technique solely is not suitable for monitoring the removal of IS [20]. Although small, IS has specific absorbance spectra in UV region and absorbance values from certain wavelengths adds specificity to the optical model created in this study.

A classical way to estimate dialysis adequacy is to estimate urea reduction ratio (URR) in % [9]. Urea describes removal of small molecules [21] and is not representative for

removal of other molecules such as the protein-bound or the middle molecules groups of uremic toxins [10]. Therefore, describing the elimination of protein bound molecules by estimating the TR_IS optically from the spent dialysate would be beneficial. Moreover, due to specific kinetic behavior of some protein bound molecules in the body, including IS, estimating the TR value on the basis of blood samples, could be misleading [10].

As seen from the Fig. 3, 4 and 5, concentration and removed amount of IS can be estimated optically. From figure 5 it is seen that TR values for urea and IS are rather different. It seems to confirm that a specific parameter is needed for estimation of removed protein bound compounds. On the other hand, there are no guidelines developed yet, how much IS or other protein bound molecules should be eliminated in relation to urea, and perhaps this new method could be useful in this development.

It can be seen from the Fig. 6 that TR values from HPLC are somewhat different compared to TR IS_Opt but the difference is not statistically significant ($p \leq 0.05$).

As seen from the Table 1 estimation of removed IS can be done by using optical information of the spent dialysate.

The clinical aim in the future is to improve an on-line monitoring system including simultaneous monitoring of the removal of markers for different clinically important groups of uremic toxins during hemodialysis. The present technical approach may help to confirm the previous knowledge and broaden the coming information about the uremic toxin, IS, removal during dialysis and a positive impact to the patients according to needs in chronic renal failure therapy [22]. The optical technique for measuring concentration and removal of different uremic toxins may give a useful, rapid and cost-effective tool for clinicians to estimate the effectiveness of dialysis procedure.

V. Conclusion

This study examined whether absorbance and fluorescence spectral data can be used for determination of the amount of removed IS during the dialysis and whether this method could be used for description of removal of IS as a marker of protein bound uremic toxins. It was found that estimation of removal of IS during the dialysis can be done by applying the optical approach developed in this study.

New clinical trials giving access to a larger amount of data of the spent dialysate for creating more specific algorithms will be issue of the next studies.

Acknowledgment

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