Removal Estimation of Uremic CVD Marker Phosphate in Dialysis Using Spectrophoto- and Fluorimetrical Signals

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Abstract— The high phosphate (P) level is considered a key player in the genesis and progression of vascular calcification in chronic kidney disease. Therefore, it is necessary to assure the sufficient removal of this uric acid retention solute during dialysis. The aim of this study was to develop models on the basis of optical signals of a particular biofluid - the spent dialysate, a waste product of kidney replacement therapy, to estimate concentrations and removal of phosphate. Eleven uremic patients from Tallinn, Estonia, were studied during altogether 42 hemodialysis treatments. Dialysate samples were collected during each treatment and analyzed at a laboratory. Ultraviolet absorbance and fluorescence spectra of the spent dialysate samples were measured. The spectral values were transformed into phosphate concentration using multiple linear regression models. Three different models were created, one used UV-absorbance values, second fluorescence values and third combined both signals. Mean phosphate concentration (mmol/l) measured in the lab was 0.299±0.141, and 0.298±0.114, 0.296±0.115, and 0.296±0.118 estimated by UV, fluorescence and combined model, respectively. Total removed (TR) amounts of phosphate were calculated using phosphate concentration values from the lab and all three models. Achieved (TR) values (mmol) were 0.21±0.112, 0.23±0.055; 0.30±0.098 and 0.29±0.098 measured in the lab and estimated by different models respectively. The results were compared regarding mean values and SD. The phosphate concentration and total removed amount values calculated using optical models did not differ from the phosphate levels and removal observed in the clinical laboratory (p>0.05). This study indicates that it is possible to estimate phosphate level and removal during the dialysis procedure using only optical signals of the spent dialysate.

Keywords— End stage renal disease, hemodialysis, phosphate, vascular calcification, optical monitoring.

I. INTRODUCTION

Around 8-10% of the adult population in the Western world suffers from kidney damage. About half of these patients also display cardiovascular disease (CVD) [1], and cardiovascular mortality accounts for ~40-50% of all deaths in patients with chronic kidney disease (CKD) stage 4 as well as patients with end-stage renal disease (ESRD = CKD stage 5) [2, 3]. Vascular calcification (VC) compromises vascular stiffness and augments vascular inflammation [4]. In patients with CKD vascular calcification (VC) is accelerated and amplified, and heralds a poor prognosis concerning CV morbidity [5-8]. VC in CKD is a complex process where the accumulation of uric toxins plays an important role. Hyperphosphatemia is considered the key player in the development of VC in CKD. Therefore, proper and sufficient removal of this uric acid retention solute is critical in the light of ESRD patients’ survival. An easy, indirect optical method for estimating the phosphate removal has been proposed earlier by Eshberg et al. [9]. This approach used UV-absorbance in a single wavelength. Also, the method for using absorbance and fluorescence to estimate phosphate level in patients’ serum has been demonstrated [10]. An additional direct method for assessing phosphate removal has been described by Michaleu et al. [11], but disposables-reagents are needed for analysis. In this paper, three different models using optical information of the spent dialysate (UV-absorbance, fluorescence or both combined) to estimate removal of phosphate during hemodialysis were studied.

II. MATERIALS AND METHODS

Eleven chronic hemodialysis patients, six females, and five males, mean age 59 ± 15 years were included in the study in Centre of Nephrology, North Estonian Medical Centre, Estonia. Each patient was followed during four midweek dialysis procedures. All sessions lasted 240 minutes; the dialysate flow was 500 and 800 mL/min, and the blood flow 300 and 350 mL/min. Two different polysulfone dialyzers, FX8 and FX1000 (Fresenius Medical Care, Germany) with the effective membrane area of 1.4 and 2.2 m², were used. The utilized dialysis machine was Fresenius 5008 (Fresenius Medical Care, Germany). During each dialysis sessions, the following samples from the drain tube of the dialysis machine were taken: 10, 60, 120 and 180 minutes after the start of the session and at the end of the session (240 min). All of the spent dialysate was collected to the dialysate collection tank. After the end of the procedure, the tank was weighted (one liter of dialys-
ate was considered equal to 1 kg. Content was carefully stirred, and one sample (Tank) was taken from it. The concentrations of phosphate in dialysate samples (P. Lab) were determined in the Clinical Chemistry Laboratory (SYNLAB, Estonia) using standardized methods. Ultraviolet (UV) absorbance measurements of the collected dialysate samples were performed over a wavelength range of 190-380 nm by a UV-Vis-NIR spectrophotometer UV-3600 (SHIMADZU, Japan) using pure dialysate as a reference. Fluorescence measurements were made over an excitation (EX) wavelength range of 220-500 nm (excitation increment 10 nm), and emission (EM) was recorded over a wavelength range of 220-500 nm. Spectrofluorophotometer (SHIMADZU RF-5301, Japan) was used for the measurements. Measurements were performed within 8 hours after each session at the room temperature (ca. 22°C). The software Panorama spectrophotometry and UV Probe E (both SHIMADZU, Japan) used for pure dialysate and presenting measured spectral values. Additional data processing was performed in Excel (Microsoft Office Excel 2007). Part of the measured values (concentration/absorbance/fluorescence) was left out from the data analysis. The exclusion criteria were abnormal value of measured phosphate concentration or recorded optical signal (absorption/fluorescence). Described errors might occur for example when sampling was performed concurrently with self-tests of the dialysis machine.

Models for estimating phosphate (P) concentration in spent dialysate using optical signals were created utilizing Statistica 9.0 (StatSoft Inc., USA). Three different models for phosphate concentration estimation were created, one using information from the measured UV-absorbance (P. UV), second using measured fluorescence (P. Flo), and the third model used both, absorbance and fluorescence spectral values (P. Comb). For creating the models, multiple linear regression (forward stepwise regression method) was employed. The dialysate phosphate concentration was set as a dependent variable, and optical signals (UV-absorbance and fluorescence) of the spent dialysate samples were set as independent variables. Each of the three models used six independent variables.

The total removed (TR) amount of phosphate was calculated as follows:

$$TR = C_{Tank} \times W_{Tank}$$

where $C_{Tank}$ is the phosphate concentration in dialysate collection tank (mmol/L) and $W_{Tank}$ is the weight of the dialysis collection tank (kg). For calculating the TR of phosphate by the optical methods, $C_{Tank}$ values calculated using optical models were used.

To determine differences between concentrations and total removed amounts from the clinical laboratory (P. Lab) and the models, the paired two sample for means Student t-test was used (p ≤0.05 was considered significant).

III. Compliance with ethical requirements

The study was performed after the approval of the protocol by the Tallinn Research Ethics Committee at the National Institute of Health Development in Estonia and conducted by the Helsinki Declaration. Informed consent was obtained from all participating patients.

IV. Results

Three multiple linear regression models were created to calculate phosphate concentration in spent dialysate samples.

![Graphs showing regression models](image)

Fig. 3 Goodness of fit of the multiple linear regression models for estimating phosphate concentration: a - UV model, b - fluorescence model, c - combined model.

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Figure 1(a-c) shows the good fits of the obtained regression models.

Table 1 provides an overview of the obtained results and presents the concentrations and total removed amounts of phosphate in mean and standard deviation values measured with the standardized laboratory methods (P_Lab) and estimated by optical models (P_UV/Flo/Comb), correlation analysis and standard errors of estimate between the methods.

Figure 2 demonstrates the differences in the individual values of a total removed phosphate from the laboratory measurements and those calculated by the different models.

![Graphs showing differences in phosphate removal](image)

**V. DISCUSSION**

The main purpose of the presented study was to produce multi-wavelength algorithms based on optical signals of a particular biofluid – the spent dialysate, for estimation of dialysate concentration of phosphate and calculate total removed amounts of phosphate during hemodialysis procedures. This study is justified by the fact that phosphate has been found to be an inducer of vascular calcification [12-14]. Also, it has been demonstrated that culturing cells in high phosphate medium induces calcification of the cells [15]. It has been suggested that keeping serum levels of phosphate under control in patients with chronic kidney disease is highly important to prevent vascular calcification and CVD [16]. Moreover, the efficiency of phosphate removal has been recommended to be monitored in addition to traditional diagnosis and assessment techniques [17].

The results in Table 1 indicate that obtained phosphate concentrations and calculated total removed amounts were not significantly different from the values from the clinical chemistry laboratory in case of any model using optical biofluid signals (UV-absorbance, fluorescence or both combined) (0.475 *p* = 0.823).

Since phosphate does not have a significant absorbance in the used wavelength region [18], it is likely that surrogate marker(s) induce the obtained good result in concentration estimation [19, 20]. Still, these surrogate markers seem follow the dialysate concentration changes of P closely. Probably we are indicating a combined effect of several chromophores and fluorophores in our model with different elimination rates and compartmental effects that result in relatively good fit for phosphate using optical signals. The latter is indicated by relatively high linear correlation values achieved (Table 1) accompanied by good model fits (Fig 1).

It can be observed from the Figure 2 that TR values estimated by different optical models have certain random
vi. Conclusions

Performed study indicates that it is feasible to assess dialysate levels and removal of phosphate using optical signals of the spent dialysate. In this way, the efficiency of each dialysis procedure can be followed continuously concerning phosphate removal. It may have a beneficial effect on prevention or slow the progression of vascular calcification in ESRD patients.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References


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