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Signature: Med Sci Monit, 2003; 9(11): CR500-504
PMID: 14586277

WWW.MEDSCIMONIT.COM
Clinical Research

Received: 2002.06.24
Accepted: 2003.06.25
Published: 2003.11.03

Dialysis adequacy, residual renal function and serum concentrations of selected low molecular weight proteins in patients undergoing continuous ambulatory peritoneal dialysis

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: none.

Summary

Background: Low molecular weight proteins (LMWP) are considered uremic toxins. There is controversy whether in peritoneal dialysis (PD) the elimination of these toxins is influenced mainly by dialysis or by residual renal function (RRF).

Material/Methods: The aim of our study was to evaluate the relationship between serum levels of selected LMWPs, dialysis adequacy, and RRF in PD patients. 27 stable subjects were studied, mean age 50±11, dialyzed for a median period of 10 months. Serum activity of acid RNA-se and alkaline RNA-se was measured by spectrophotometry, and serum alpha$_1$-microglobulin (α_1M) concentration by ELISA. Kt/V and weekly creatinine clearance (wCl_{Cr}) were assessed as adequacy indices (both as the sum of renal and dialysis components) and RRF as the mean of residual urea and creatinine clearances.

Results: Significant inverse correlations were found between RRF and α_1M level, as well as alkaline RNA-se activity ($p<0.0001$). A similar relationship was found for residual Kt/V ($p<0.0001$ for α_1M and alkaline RNA-se). There was no significant correlation between acid RNA-se activity and any tested parameter of adequacy. When the cutoff points of wCl_{Cr} = 60 L/week/m^2, total Kt/V = 2.0, or RRF=2.0 ml/min were used, we found α_1M level and alkaline RNA-se activity to be significantly lower in patients with higher values of the respective parameters.

Conclusions: RRF plays an important role in elimination of LMWP in PD. The activity of alkaline RNA-se and acid RNA-se behaves differently in these patients.

key words: uremic toxins • peritoneal dialysis • acid RNA-se • alkaline RNA-se

Full-text PDF: http://www.MedSciMonit.com/pub/vol_9/no_11/2878.pdf
Word count: 2101
Tables: 3
Figures: –
References: 28

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BACKGROUND

Uremic toxemia occurs due to the effect of catabolite accumulation, which adversely affects the function of various organs and tissues. These substances may be divided into several categories. According to their molecular weights, they are classified as low (<300), middle (300–15,000) and high molecular weight uremic toxins (>15000) [1,2]. The urea and creatinine concentrations or urea and creatinine elimination rates (clearances) are generally used as a common denominators of uremic toxin accumulation. The same indices are used to assess dialysis adequacy. Though a great amount of data has demonstrated a relationship between the patients' clinical outcome, normalized clearance of urea (Kt/V), and weekly creatinine clearance (Cl_{Cr}) [3–6], the importance for the development of toxemia of many individual compounds accumulating in the plasma of end-stage renal disease patients is still unknown. Thus particular interest has been focused on the group of low molecular weight proteins (LMWPs) accumulating in plasma, along with gradual loss of renal function, remaining extremely elevated in patients maintained on renal replacement therapy [7–11]. As shown by Jung et al., the accumulative increase of plasma ribonuclease is a more sensitive indicator of renal function impairment than creatinine [12]. A decrease in glomerular filtration is the most common cause of elevated plasma α_1 microglobulin (α_1M). Others authors suggest that the measurement of α_1M may be helpful in the so-called creatinine-blind range of glomerular filtration rate (GFR) decrease. α_1M is probably superior to β_2-microglobulin or retinol-binding protein to measure loss of renal function, although probably cystatin C elimination measurement would be best for this purpose [13]. The elimination of α_1M with urine also appears to be a useful diagnostic tool of subclinical nephropathy, as a marker of early renal damage, for example in patients with stable angina without symptoms of kidney disease [14]. On the other hand, serum β_2-microglobulin concentration is related not only to the current level of renal function, but also to many pathologies associated with inflammatory process or cellular damage, since this protein is one of the domains of histocompatibility class I HLA antigens [15,16].

The LMWP accumulation process may serve as a model of uremic toxin accumulation independently of the urea and creatinine removal rate. Urea and creatinine elimination are assessed in the evaluation of dialysis adequacy because of the simplicity, but the serum levels of these solutes do not reflect the state of toxemia, as they are not toxic themselves. It would be much more appropriate to assess other compounds of larger molecular weight, but such methods have not been developed as routine procedures for everyday clinical practice.

The aim of our study was to make a cross-sectional measurement of serum levels of selected LMWPs in patients on continuous ambulatory peritoneal dialysis (CAPD), and to evaluate the potential impact of residual renal function and dialysis adequacy on serum levels of these proteins. The most important issue in this study was to assess the contribution and importance of both residual diuresis and peritoneal dialysis to the elimination of LMWPs.

MATERIAL AND METHODS

27 clinically stable CAPD patients were enrolled into the study: 13 females, 14 males, ranging in age from 22 to 74 years (mean 50±11 years) dialyzed for a median period of 10 months (range 1–56 months). The etiology of end–stage renal disease included primary glomerulopathy in 13 patients, polycystic kidney disease in 2, chronic pyelonephritis in 1 and amyloidosis in 1. In 10 cases no primary diagnosis of chronic nephropathy had been established, mainly due to late referral to the renal unit.

CR

Samples for RNA-ses were centrifuged at 1000 g, 4°C. RNA-se activity was determined from 20-μl serum samples incubated with 180 μl of 100 mmol/l sodium phosphate buffer (8.5 ml of 0.2 M. NaH_2PO_4 plus 91.5 ml of 0.2 M. Na_2HPO_4 – pH 7.8 for alkaline RNA-se or 68.5 ml of 0.2 M. NaH_2PO_4 plus 31.5 ml of 0.2 M. Na_2HPO_4 – pH 6.5 for acid RNA-se), plus 200 μl of RNA substrate (6 g/l RNA in sodium phosphate buffer, pH 7.8 for alkaline RNA-se or pH 6.5 for acid RNA-se). After 60 minutes of incubation the RNA precipitating agent (600 μl of 6 mmol/l uranyl acetate) was added. The samples were centrifuged at 4°C. 100 μl of clear supernatant was taken off and diluted in 1.4 ml of distilled water. Then spectrometric absorption at 260 nm was determined. A blank assay was prepared exactly as the samples, omitting the incubation step. Each test was performed in duplicate, and final RNA-se activity was calculated as a mean value.

All values were expressed in arbitrary units. One unit of RNA-se activity was the amount of hydrolized RNA which under standard conditions liberates low molecular RNA-degradation products ($A_{260}=0.360\times cm^{-1}$). RNA-se activity was calculated by equation (1):

$$RNA\text{-}se\ (U/l) = [(A_{260}-0.02)/0.316]^2 \quad (1)$$

This equation shows the relationship between RNA-se concentration and the increased light absorption at 260 nm.

Acid and alkaline RNA-se isoenzymes were assayed by determining the total RNA-se activity at pH 6.5 ($RNA\text{-}se_{6.5}$) and pH 7.8 ($RNA\text{-}se_{7.8}$), and then further calculations were performed using equations (2) and (3):

$$alkaline\ RNA\text{-}se = 1.11 \times RNA\text{-}se_{7.8} - 0.67 \times RNA\text{-}se_{6.5} \quad (2)$$

$$acid\ RNA\text{-}se = RNA\text{-}se_{6.5} - 0.17 \times alkaline\ RNA\text{-}se \quad (3)$$

The origin of all three equations used here has been described in detail elsewhere [10,17].

α_1M was determined in serum using the nephelometric method (Behring Nephelometer, Behringwerke AG, Marburg, Germany). Intra-assay variability for this assay was less than 5%.

Dialysis adequacy was assessed using the sum of dialysis and residual urea and creatinine clearances, standardized to distribution volume (Kt/V) or body surface area

Table 1. The mean/median values and SD/range of the serum levels/activity of the tested low molecular-weight proteins, and dialysis adequacy parameters.

	Unit	Mean/Median	SD/Range
α_1M	*mg/l*	29.4	14.8
Acid RNA-se	*U/l*	0.29	0.038–0.78
Alk. RNA-se	*U/l*	1.56	0.42
wCl_{Cr} total	*l/week/1.73m²*	69.5	40.0–127.1
wCl_{Cr} dial.	*l/week/1.73m²*	37.6	9.9
Cl_{Cr} res	*ml/min*	2.81	0–9.24
Kt/V total	–	1.96	0.35
Kt/V dial.	–	1.4	0.39
Kt/V res.	–	0.63	0–1.7
RRF	*ml/min*	2.29	0–7.07

Abbreviations: as in the text

(wCl_{Cr}). Serum, urine and dialysate urea and creatinine levels were measured using a Hitachi 917 analyzer. Urine and dialysate urea and creatinine were measured in 24-hour collections. Fasting blood was taken in the morning the day after collection was completed. Adequacy indices (Kt/V, wCl_{Cr}) were calculated using the licensed, commercially available software package Nephron for Windows (DDPS, Cracow, Poland). This program utilizes anthropometric Watson formulas for distribution volume calculations. RRF was expressed as the mean of the sum of residual renal urea and creatinine clearances. In addition, the dialysate-to-plasma creatinine ($D/P_{Creat.}$) ratio in the standardized Peritoneal Equilibration Test (PET) was calculated for every subject, according to the method described by Twardowski [18].

Statistical analysis

Statistical analysis of the data was performed with Statistica 5.1 software (StatSoft Inc, Tulsa, Oklahoma, USA). Shapiro-Wilk's *W*-test of normality was used for data distribution analysis. All variables with normal distribution were presented as mean ±SD; those with non-normal distribution, as median and range. The t-Student test for inter-group comparisons and Pearson test for correlations between variables were used for normally distributed data, whereas the *U* Mann-Whitney and *R* Spearman test, respectively, were applied to non-normally distributed data. We considered a p value less than 0.05 to be statistically significant.

RESULTS

The parameters analyzed in the present study are summarized in Table 1. The median value of $D/P_{Creat.}$ was 0.56 (range 0.35–0.8), with 14 patients classified as Low/Low-Average Transporters (L/LA, median 0.51; range 0.35–0.56), and the remaining 13 as High/High-Average Transporters (H/HA, median 0.76; range 0.68–0.8).

Significant negative correlations were demonstrated for RRF and the α_1M level, as well as RRF and alkaline RNA-se serum activity ($R=-0.83$; $p<0.0001$ and $R=-0.73$; $p<0.0001$, respectively; *R* Spearman rank test). A similar relationship was found for residual Kt/V ($R=-0.48$; $p<0.0001$ for α_1M and $r=-0.79$; $p<0.0001$ for alkaline RNA-se). Inverse relationships were also found for the level/activity of both mentioned proteins and residual diuresis volume ($R=-0.76$; $p<0.0001$ for α_1M and $R=-0.69$; $p<0.0001$ for alkaline RNA-se), as shown in Table 2.

The total values of both wCl_{Cr} and Kt/V depended strongly on residual components of these adequacy parameters ($r=0.88$; $p<0.0001$ for total and residual wCl_{Cr}, and $r=0.64$; $p<0.005$ for total and residual Kt/V), whereas no relationship was observed between total and dialytic components.

No statistically significant correlation was found between acid RNA-se activity and any tested parameter of adequacy. It is worth nothing that there were positive correlations between α_1M, alkaline RNA-se and dialytic components of both analyzed adequacy parameters. α_1M serum level and alkaline RNA-se but not acid RNA-se activity were proportional to dialysis treatment duration (Table 2).

Dividing our patients according to the cutoff values of a total wCl_{Cr} of 60 L/week/m², total Kt/V of 2.0 or RRF of 2.0 ml/min, we found α_1M serum level and alkaline RNA-se activity to be significantly lower in patients with values above the respective cutoff lines (Table 3). No differences were found in serum concentration or activity of tested LMWPs between L/LA vs. H/HA transporters.

DISCUSSION

The molecular weight of serum RNA-ses fits in the range between 8,500 and 33,000, whereas the molecular weight of α_1M is equal to 30,000. In comparison, β_2-microglobulin, the protein usually used to assess the accumulation of larger molecular size toxins, has a molecular weight of 11,800. Thus the analyzed proteins should be classified into the group of toxins with the largest molecular size [1].

The results obtained in this study indicate that serum concentrations of the selected low molecular weight proteins directly depend upon residual diuresis. Both α_1M level and alkaline RNA-se activity correlated inversely with the values of total and residual components of both tested indices of adequacy (residual Kt/V and wCl_{Cr}). Differences in elimination of substances with higher molecular weight in dialyzed patients with preserved residual diuresis in comparison to anuric individuals have already been demonstrated for β_2-microglobulin [19,20]. Not only β_2-microglobulin, but also RNA-se seem to be good markers of decreasing glomerular filtration rate [12,21]. It has been suggested that RNA-se may be a very useful and sensitive indicator of decreased renal function in patients who still maintain normal creatinine clearance [12].

Interestingly, serum levels of α_1M and alkaline RNA-se correlated directly with the dialytic components of both analyzed adequacy indices. This phenomenon may be

Table 2. Correlation between α_1M serum level, alkaline RNA-se activity and parameters of dialysis adequacy (*R Spearman rank test, unless stated otherwise*).

		CAPD duration	RRF	Kt/V dial.	Kt/V res.	Kt/V total	wClCr dial.	wClCr res.	wClCr total
α_1M	R=	0.57	–0.83	0.64	–0.84	–0.62	0.66	–0.82	–0.64
	p<	0.01	0.0001	0.001	0.0001	0.005	0.001	0.0001	0.005
Alk.	R=	0.57	–0.73	0.46*	–0.79	–0.63*	0.39*	–0.84	–0.64
RNA-se	p<	0.01	0.0001	0.05	0.0001	0.001	0.05	0.0001	0.05

* Pearson's test;
Abbreviations: as in the text

Table 3. Comparison of serum level/activity of tested LMWPs among patients with higher and lower values of total wClCr, Kt/V and RRF.

	α_1M (mg/l)	Alk. RNA-se (U/l)
wCl_{Cr} total >60 (*L/week/1.73m²*)	19.45 (11.6–32.3)	1.32±0.28
(N=14)		
wCl_{Cr} total <60 (*L/week/1.73m²*)	43.9 (15.7–60.6)	1.82±0.41
(N=13)		
	p<0.01*	p<0.001**
Kt/V >2.0	19.0 (11.6–53.5)	1.32±0.32
(N=13)		
Kt/V <2.0	39.4 (27.3–60.6)	1.78±0.39
(N=14)		
	p<0.005*	p<0.005**
RRF >2.0 (ml/min)	18.5 (11.6–32.3)	1.34±0.28
(N=14)		
RRF <2.0 (ml/min)	43.9 (27.3–60.6)	1.84±0.38
(N=13)		
	p<0.001*	P<0.0005**

* *U* Mann-Whitney test;
** *t*-Student test;
Abbreviations: as in the text

explained by the well-known fact that the dialysis dose increases following RRF decline, as do the dialytic components of Kt/V or wCl_{Cr}. Our findings may suggest that an increased dialysis dose cannot replace residual renal function in terms of LMWP removal.

As shown in our study, acid RNA-se behaves in a different mode compared to alkaline RNA-se. This may be explained by the differing origin of these two enzymes. Acid RNA-se is mainly derived from leukocytes, and its release during dialysis may reflect bioincompatibility of renal replacement therapy, as has been shown for hemodialysis (HD) [22,23]. It has been demonstrated that a HD session leads to increased activity of different enzymes, including acid RNA-se, mainly due to leukocyte degranulation on dialysis membranes [11,24]. On the other hand, alkaline RNA-se is predominantly a pancreatic enzyme, and its release is probably less influenced by dialysis itself, hence the effect of its removal may be demonstrated. CAPD is a continuous mode of dialysis with relatively steady-state levels of various solutes. However, the continuous stimulation of leukocytes by bioincompatible dialysis fluid resulting in the prolonged release of leukocyte enzymes (with a rate comparable to their elimination) may be taken under consideration.

Many data indicate that residual renal function is important for obtaining the desired values of dialysis adequacy indices, and its impact on the clinical outcome of renal replacement therapy is well documented [25–27]. Contemporary methods of dialysis allow replacement of reduced kidney function. It is beyond doubt that an increasing dialysis dose allows for efficient elimination of small solutes to an extent compensating for deteriorating renal function. It seems, however, that this treatment cannot compensate for lost renal elimination of substances with higher molecular weights, responsible for the development of uremic toxemia. This is due to the lack of the fundamental metabolic function of the kidneys in the elimination of these substances, which also includes protein catabolism. In this respect, the question as to whether deteriorating renal function can be replaced with increasing dialysis dose remains open.

Based on the results of the latest large-scale prospective studies, which failed to demonstrate any substantial impact of small solute clearance on long-term survival, we believe that the assessment of the serum concentration of substances with larger molecular weight appears to be more appropriate to describe uremic toxemia [28].

Conclusions

The results of our study seem to indicate that serum levels or enzymatic activity of the selected small-molecule proteins depend on residual renal function. This means that the assessment of peritoneal dialysis efficacy by reference to the elimination of these substances alone, particularly among patients without residual renal function, does not reflect the actual accumulation of toxic metabolites. It should be noted that the behavior of acid and alkaline RNA-se activity is different, with no relationship between acid RNA-se and RRF or dialysis adequacy.

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