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Use of spent dialysate analysis to estimate blood levels of uraemic solutes without blood sampling: urea

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Abstract

Background. Urea kinetic modelling-based methods are widely used to assess dialysis efficacy. However, they require blood sampling and are susceptible to a number of errors, mainly from the calculated parameters (particularly *V*). Spent dialysate determinations have been used and have been shown to be reliable and simple to use. In this study, we associated dialysate-based and clear-

ance determinations along with Kt/*V* to estimate blood urea levels.

Methods. Urea kinetic modelling, continuous sampling of spent dialysate and ionic dialysance were determined in 18 stable dialysis patients during 126 dialysis sessions. Mean blood urea levels were estimated as follows: mean urea level = spent dialysate − urea mass/(dialysance * *T*). Blood urea levels before and after dialysis were calculated based on the same determinations and extended formulae.

Results. Estimated mean urea level was significantly correlated with measured mean blood urea level ($R^2 = 0.957$; $P < 0.0001$), and Bland and Altman analysis showed significant agreement between estimated and measured levels. Estimated and measured blood urea levels were also correlated before and after dialysis ($R^2 = 0.972$, $P < 0.0001$ and $R^2 = 0.903$, $P < 0.0001$, respectively), with good agreement for both blood urea before and after dialysis and their respective estimates.

Conclusions. Blood urea levels may be reliably estimated from the total mass of urea removed in the dialysate and the dialysance measured during dialysis. Coupling both measurements allows a precise monitoring of dialysis efficacy and a specific evaluation of the patient's urea metabolism status. Technical dysfunctions and patient variations may be easily identified using this approach without blood sampling.

Keywords: dialysance; haemodialysis; spent dialysate; uraemic solute determination

Introduction

Following the classical work by Sargent *et al*. [1], urea kinetic modelling (UKM) [2] has been adopted as the most common method to assess dialysis delivery. After an initial period marked by the National Cooperative Dialysis Study (NCDS) in the early 1980s [3], the tendency has been to increase the target Kt/*V*, which is presently commonly acceptable at >1.2. Guidelines have been built on these recommendations [4]. However, UKM-based methods are susceptible to errors [5], mainly in the calculated parameters, such as the urea distribution volume *V* [6]. Alternative methods have been proposed to avoid these errors, the simplest one being the use of Kt without *V* [7]. Other methods have been based on partial dialysate collection [8], which has evolved to continuous sampling of spent dialysate (CSSD) [9]. This technique, which does not require blood determinations, has been proven to be reliable and easy to apply in clinics. Several groups have reported on the removal of different compounds of clinical interest (proteins, phosphate, calcium, creatinine and urea, among others) by dialysis using this system [9–12]. In addition to the measurement of total mass removal, clearance may also be determined during dialysis. Urea clearance surrogates have been proposed, and particularly total ionic dialysance by measurement of dialysate conductivity, based on the methods first proposed by Albers and Smith [13] and Polaschegg [14], has been used and applied to *in vitro* and *in vivo* dialysis quantification [15,16]. This method is also simple and reliable and does not require blood determinations. However, in clinical practice, serum urea levels are by far preferred to either mass removal or clearance of urea, as they are more meaningful to the physicians in charge of the dialysis patients.

In the present study, we aimed to combine both CSSD and ionic dialysance to assess dialysis dose and to estimate blood urea levels. We show that the combination of CSSD and dialysance is also sensitive and reliable in estimating blood levels of urea in stable dialysis patients. Therefore, these dialysate-based techniques may be used to monitor urea metabolism, including blood urea level estimation without blood sampling.

Table 1. Patient demographics and characteristics

	Mean	SD	Units
Patients			
Age	79.3	6.8	years
Height	163	8	cm
Weight after dialysis	66.2	10.1	kg
Total body water (from blood values)	34.1	6.0	L
Total body water (from Watson *et al*. formula)	33.7	3.4	L
Total body water (from Chumlea formula)	36.7	4.2	L
Dialysis			
Time	222.4	26.7	mn
Qb	317.7	22.5	mL/mn
Qd	500	10	mL/mn
Weight loss	2.6	1.1	kg
Number of dialysance measurements per session	3.6	1.1	
Number of sessions	144		
Number of valid sessions	126		

Materials and methods

Eighteen stable dialysis patients treated in the dialysis centre of Néphrologie Dialyse St Guilhem in Sète were included in the study. They were dialysed three times a week with fully equipped AK200S machines (Gambro, Lund, Sweden) using ultrapure bi-reverse osmosis water. They had been on dialysis for more than 3 months and had no active disease at the time of the analyses. They were able to understand the study and gave informed consent to participate in it following the informed consent sheet approved by the Comité de Protection des Personnes of Nîmes (2009.01.07 bis) with the registration number at the French Agency AFSSAPS 2008-A01612-53, which they signed. High permeability dialysers (XEVONTA 2.3, B BRAUN, Melsungen, Germany; FX100, 2.2 m^2, Fresenius AG, Bad Homburg, Germany; ELISIO 210, NIPRO Co, Japan; XENIUM 210, Baxter Co, McGaw Park, Illinois USA) were used. Their characteristics are described in Table 1.

Sample collection and analyses

Spent dialysate was continuously sampled with a reversed injection pump throughout the complete dialysis procedure as previously described [9]. A sample of the pulled dialysate was used for urea determination. Blood was sampled on the arterial dialysis tubing before and after dialysis (with stopped blood pump).

Urea was determined by UV kinetic analyses with an AU 400 automate (Olympus, Hamburg, Germany). The linearity range was from 0.8 to 50 mmol/L and the sensitivity threshold 0.38 mmol/L, with a variation coefficient for our laboratory of 1.8%. All the values observed in this study were within the linearity range of the method used (1.5 mmol/L the lowest observed value in spent dialysate and 31.5 mmol/L the highest in blood).

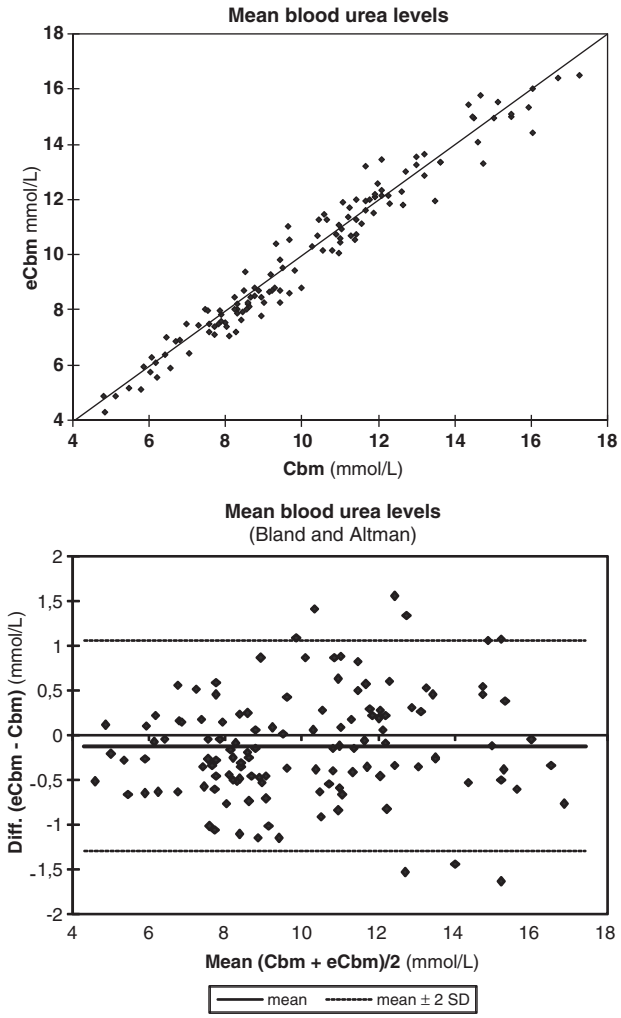

Fig. 1. Correlation and differences between estimated (eCbm) and measured mean blood urea levels (Cbm).

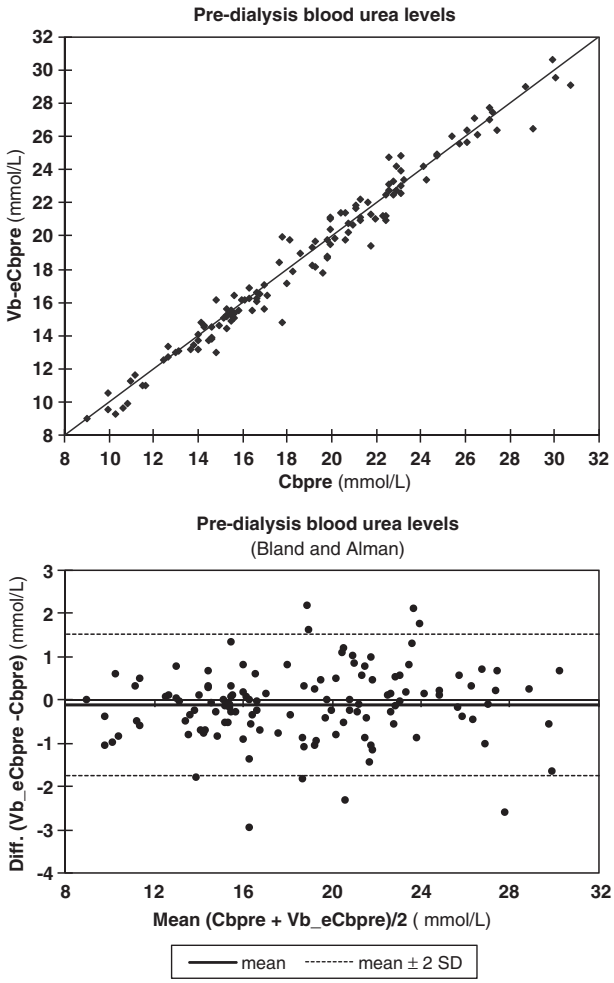

Fig. 2. Correlation and differences between measured and predialysis blood urea levels (Cbpre) estimated with urea distribution volume calculated from blood values (Vb_eCbpre).

Blood urea level estimation from dialysate measurements

Blood urea levels were estimated from urea measurements in spent dialysate. The formulae used to obtain the estimated values are given here and their fundamentals are detailed in Appendix 1.

Mean blood urea concentration (eCbm) eCbm = Murea/Durea * T

Pre-dialysis blood urea concentration (eCbpre) eCbpre = eCbm * $K * T/V/(1 - \exp^{(-KT/V)})$

Post-dialysis blood urea concentration (eCbpost) eCbpost = eCbpre * $\exp^{(-KT/V)}$

Statistics

The values of the different variables are given as mean ± standard deviation of the mean (unless otherwise specified). Differences between estimated and measured values were tested using a Student's *t*-test for paired data. Both measured and estimated blood urea levels were assessed for correlation (Pearson's *R* value) and Bland and Altman [17] analyses. If correlation studies were considered significant, linear regression analyses were performed. Bland and Altman analyses tested the agreement between the measured and estimated values. *P* values <0.05 were considered significant.

The analyses were performed using the SAS statistical package V 9.1 (SAS Corporation, Cary, NC, USA)

Results

Estimated mean blood urea levels

Measured mean urea blood level was 10.17± 2.83 mmol/L (range 4.78 to 17.26 mmol/L), and estimated mean blood urea level from the dialysate measurement was 10.05 ± 2.91 mmol/L (range 4.31 to 16.49 mmol/L) (NS). Both variables were significantly correlated ($R^2 = 0.957$; $P < 0.0001$), and Bland and Altman [17] analysis showed significant agreement between estimated and measured mean blood urea levels (average differences = −0.12 ± 0.60 mmol/L; Figure 1).

Estimated pre-dialysis blood urea levels

There were no significant differences between measured and estimated pre-dialysis blood urea levels regardless of the method used for *V* determination [18.83 ± 4.95 mmol/L vs 18.72 ± 5.03, 18.84 ± 5.30 and 18.05 ± 5.10 for blood

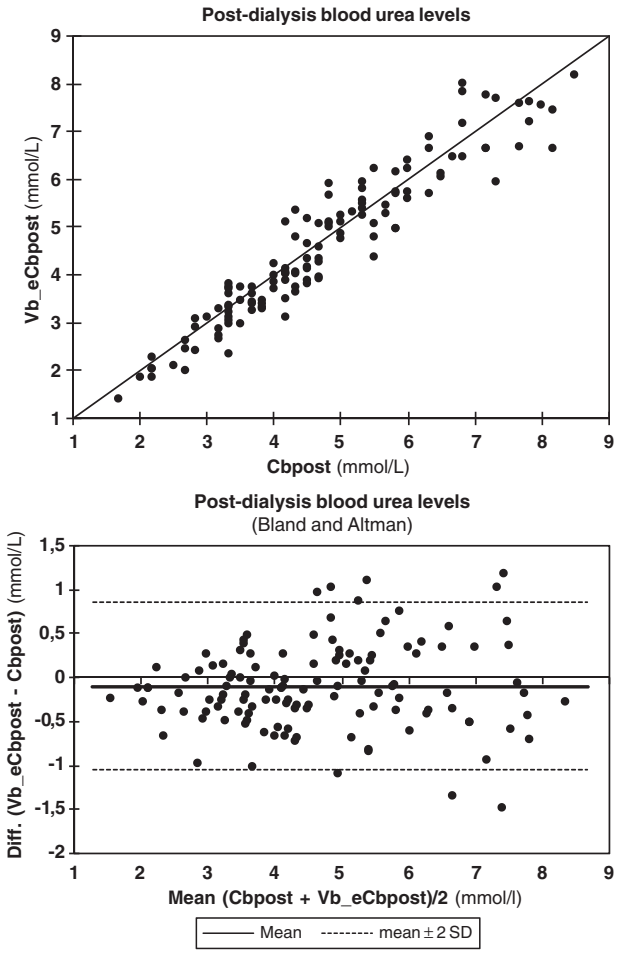

Fig. 3. Correlation and differences between measured and post-dialysis blood urea levels (Cbpost) estimated using urea distribution volume calculated from blood values (Vb_eCbpost).

derived using the NCDS [3], Watson [18], and Chumlea formulas [19] respectively (NS)]. Mean differences between estimated and measured blood urea concentration were −0.11 ± 0.84, 0.01 ± 1.36 and −0.78 ± 1.16 mmol/L, respectively (NS).

Estimated and measured Cbpre were significantly correlated. The correlation analyses showed an $R^2 = 0.972$ (Figure 2), $R^2 = 0.935$ and $R^2 = 0.949$ ($P < 0.0001$) for the estimated values, respectively, using blood determinations or the Watson as well as Chumlea formula in obtaining *V*.

Bland and Altman analysis showed significant agreement in the three situations; Figure 2 illustrates the results with the NCDS' derived *V* calculation.

Estimated post-dialysis blood urea levels

There were no significant differences between measured post-dialysis blood urea levels (4.71 ± 1.53 mmol/L) and those estimated using blood determinations (4.61 ± 1.58 mmol/L), the Watson (4.58 ± 1.52 mmol/L) or Chumlea (4.89 ± 1.62 mmol/L) formula. Mean differences between estimated and measured blood urea concentrations were −0.10 ± 0.49 mmol/L, −0.138 ± 0.61 and 0.18 ± 0.59 mmol/L, respectively.

Estimated and measured Cbpost were significantly correlated. The correlation analyses showed an $R^2 = 0.903$, $R^2 = 0.849$ and $R^2 = 0.868$ ($P < 0.0001$) for the estimated values, respectively, using the NCDS, Watson or Chumlea formulas in obtaining *V*.

Bland and Altman analysis showed significant agreement in the three situations; Figure 3 illustrates the results with the NCDS-derived *V* calculation.

Discussion

Following the main aim of delivering the adapted dose of dialysis to end-stage renal disease patients, best practice guidelines have emerged, which recognize total dialysate collection as the gold standard method for urea kinetic analysis [20,21]. In addition to allowing a mass quantification of the substances to be removed from the patient, this method minimizes the exposure of patients and staff to blood-borne pathogens. However, since total dialysate collection is impractical in the clinical setting, it has not been adopted in dialysis units, and many of the guidelines are still focussed on blood-based measurements of urea removal. Kt/*V* obtained from blood measurements remains the most widely used approach to assess dialysis adequacy despite its widely acknowledged limits.

We previously reported on the accuracy of using CSSD as a dialysate-based alternative method to total dialysate collection and proposed it for clinical use [9]. However, in clinical practice, physicians are more familiar with serum levels of urea than with mass removal or urea clearance. In the present study, we wanted to estimate blood values from spent dialysate-derived measurements trying to extend our previous approach and provide reference figures to the commonly used blood urea levels, still minimizing the blood-linked drawbacks. Our results remarkably demonstrate the accuracy of the estimated parameters and the agreement existing between estimated and blood-measured levels of urea.

Abundant literature has dealt with the limits of Kt/*V* obtained from blood measurements [5]. The low reliability of a urea clearance obtained from a single point during dialysis, the high variability observed on the different parameters during the dialysis session and particularly the inaccurate determination of the distribution volume of urea (*V*) are the most prominent ones [6,26]. To reduce the error in determining *V*, specific methods have been proposed; among them, multi-frequency bioimpedance analysis (BIA) appears as a non-invasive method, which would be more accurate than anthropomorphic-based formulas [27,28].

We wanted to focus in this study on the benefits of the dialysate-based methods to overcome most of the drawbacks of the blood-based determinations mentioned above. The advantages of CSSD include easiness to run and limited volume to handle. Most importantly, CSSD allows a precise quantification of removal, overcoming the inaccuracies linked to *K* variation, determining the volume of distribution of urea or access recirculation. Measuring the total removal over the dialysis session avoids the variations occurring during the treatment and does not require *V* determination.

Partial dialysate collection is also useful to assess protein catabolic rate [8]. An increased serum urea level may be the consequence of an increase in protein intake or, in unstable patients, an increase in protein catabolic rate. The association of CSSD and ionic clearance allows for the identificatin of variations in K that should warn about decreased dialysis performance, particularly due to vascular access dysfunction: diminished vascular access flow and increased recirculation.

In conclusion, blood urea levels may be reliably estimated from the total mass of urea removed in the dialysate by associating CSSD and the dialysance measured during dialysis. Coupling both techniques provides a new method to assess dialysis adequacy that not only precisely evaluates removal but may also be oriented to patient's status (anabolic- or catabolic-dominating state or vascular access dysfunction), which still does not require blood determination and is not susceptible to most of the errors observed in blood-based UKM calculations. Although this method will not completely avoid blood sampling (still needed to periodically check blood cell counts as well as serum levels of albumin, calcium and other molecules), it allows for the assessment of urea metabolism on a more regular basis without blood sampling. This method may be adapted to other uraemic retention solutes.

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Appendix 1

Estimation of mean blood urea concentration from dialysate measurements

Reference value

Blood urea levels were determined before and after dialysis. The mean urea level obtained from blood measurements (Cbm) was calculated as follows:

$$\text{mean urea level} = \text{Cbm} = (\text{Cbpre} - \text{Cbpost})/\ln(\text{Cbpre}/\text{Cbpost}). \quad (1)$$

Estimated value of mean blood urea level

The mean urea levels in the blood were estimated (3) from the dialysate determinations according to the following equations:

$$K \text{ urea} = \text{Murea}/(\text{Cbm} * T)$$

where K is the clearance, Murea is the total mass of urea in the dialysate and T is the time of dialysis.

K can be obtained by measuring dialysance during dialysis, which will be a function of the ionic dialysance as previously reported [14,22–24]:

$$K \text{ urea} = D \text{ Urea} = f(\text{total ion dialysance})$$

Then

$$\text{Durea} = \text{Murea}/(\text{Cbm} * T)$$
$$\text{Cbm} = \text{Murea}/(\text{Durea} * T)$$

Therefore, Cbm may be obtained without measuring blood levels, knowing the total mass removed in the dialysate, the dialysance and the time of dialysis. For the purpose of differentiating from the Cbm obtained from blood measurements, we will call the estimated mean urea concentration **e**Cbm, which will be calculated as described.

$$\mathbf{e}\text{Cbm} = \text{Murea}/(\text{Durea} * T) \quad (2)$$

Murea may be obtained by measuring the concentration of urea in CSSD [9] and multiplying by dialysate flow and time of dialysis, as follows:

$$\text{Murea} = \text{Cdurea} * (\text{Qd} * T + \text{WL})$$

where

Cdurea = concentration of urea in spent dialysate

Qd = dialysate flow in L/mn (it remained constant during session)

T = dialysis time

WL = weight loss

Estimation of pre- and post-blood urea levels from dialysate measurements

Reference value

The reference values were those obtained by direct measurement both before and after dialysis (Cbpre and Cbpost).

Estimated pre- and post-dialysis blood urea levels

The pre- and post-blood urea levels were estimated from the dialysate determinations based on urea kinetic modelling [25] as follows:

$$K * T/V = \ln(\text{Cbpre}/\text{Cbpost}) \quad (3)$$

and

$$\text{Cbpost} = \text{Cbpre} * \exp^{(-KT/V)} \quad (4)$$

If we replace, in equation (1), ln (Cbpre/Cbpost) by $K * T/V$ (3)

$$\text{Cbm} = (\text{Cbpre} - \text{Cbpost})/K * T/V$$
$$\text{Cbpre} - \text{Cbpost} = \text{Cbm} * K * T/V$$

From equation (4) $\text{Cbpre} - \text{Cbpost} = \text{Cpre} - \text{Cbpre} * \exp^{(-KT/V)} = \text{Cbm} * K * T/V$

$$\text{Cbpre}*(1 - \exp^{(-KT/V)}) = \text{Cbm} * K * T/V$$
$$\text{Cbpre} = \text{Cbm} * K * T/V/(1 - \exp^{(-KT/V)})$$

Cbm can be estimated by equation (2)

$$\text{Cbpre} = \text{eCbm} * K * T/V/(1 - \exp^{(-KT/V)})$$

Knowing Cbpre, Cbpost can be calculated with equation (4).

Therefore, Cbm, Cbpre and Cbpost may be estimated, without measuring blood levels, from the total mass removed in the dialysate, the dialysance D instead of clearance (K), the time of dialysis and the urea distribution volume V. For the purpose of differentiating from the Cbpre and Cbpost obtained from blood measurements, we will call the estimated urea concentration in blood eCbpre and eCbpost.

Estimated blood volume urea (Vurea)

Three different methods for urea distribution volume were included in the study and checked for accuracy.

eCbpre and eCbpost were calculated by using three different equations to estimate urea.

1. UKM (3) -based estimation of urea distribution volume (VUKM)

$$K*T/V=\ln (\text{Cbpre}/\text{Cbpost})$$

Replacing K by the dialysance

$$D * T/V=\ln (\text{Cbpre}/\text{Cbpost})$$

Therefore:

$$\text{VUKM} = D * T/\ln (\text{Cbpre}/\text{Cbpost})$$

V1 was calculated for all the dialysis sessions included in the study. The average values per patient were taken to estimate eCbpre and eCbpost.

2. Anthropometric formula of Watson *et al.* [18] taking into account age, height and weight:
V_{WAT} for men = 2.447 − 0.09516 * age + 0.1074 * height + 0.3362 * weight
V_{WAT} for women = 2.097 + 0.1069 * height + 0.2466 * weight

3. Anthropometric formula Chumlea *et al*. [19] taking into account age, height, weight and race:
V_{CHU} for white men = 23.04 − 0.03 * age + 0.5 * weight − 0.62 * weight/(0.01 * height)^2
V_{CHU} for white women -= − 10.5+ 0.01 * age + 0.2 * weight + 0.18 * height

Conflict of interest statement. All the authors declare to have no conflict of interest in regards with the data contained in the submitted work.

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Oxidative DNA damage in chronic renal failure patients

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Abstract

Background. Chronic renal failure (CRF) patients present a high incidence of cardiovascular pathologies and cancer. This has been attributed to the existence of genomic instability in these patients, and consequently they should present elevated levels of genetic damage.

Methods. To determine the background levels of genetic damage and its specific levels of oxidative damage, a large population of 253 CRF patients (77 in dialysis) was analysed using the comet assay. The percentage of DNA in the tail was used as a measure of basal genetic damage. In addition, the use of endo III and FPG enzymes allowed us to determine the levels of specific oxidative damage in DNA bases.

Results. This is the first study that uses endo III and FPG enzymes to measure oxidative damage in CRF patients. Overall genetic damage, as well as specific oxidative damage, was higher in dialysis patients than in the CRF patients with different stages of uraemic state; genetic damage increased when serum creatinine levels increased. Genomic damage in dialysis patients decreased in those patients submitted to dialysis for a long time.

Conclusions. Genetic damage increases when renal function decreases, being maximum in haemodialysis patients. Although part of the observed damage can be attributed to the uraemic state itself, other individual genetic factors can influence a state of genomic instability responsible for the observed genomic damage.

Keywords: comet assay; CRF; haemodialysis treatment; oxidative damage; uraemic state

Introduction

The reactive oxygen species released by the mitochondrial respiratory chain can damage biomolecules such as lipids, proteins and nucleic acids. To avoid the damage, antioxidant defences have evolved to remove most of these oxidant agents. Even if a balance between oxidative damage and protective mechanisms is usually kept, there are specific situations in which the excessive production of free radicals, or deficiencies in antioxidant defences, leads to the appearance of oxidative stress [1].

Evidence exists indicating that end-stage renal disease is associated with oxidative stress, as a result of both increased oxidant production and decreased antioxidant defences [2–4]. In addition, patients with end-stage renal disease have been reported as a group with a high incidence of cardiovascular disease and cancer [5–7], and this could be related to increased levels of genetic damage.

Chronic kidney disease is a pathology characterized by progressive impairment of renal function over time , the glomerular filtration rate (GFR) being the best measure of kidney function. The early stages of chronic kidney disease (stages 2 and 3) are manifested by mild to moderate decreased glomerular filtration rate and are generally asymptomatic; but its diagnosis is important to treat cardiovascular risk factors, to delay progression of chronic kidney disease and to prevent cardiovascular events. Advanced stages of chronic renal disease (4 and 5) are characterized by severely decreased glomerular filtration rate accompanied by clinical complications (hypertension, anaemia, bone disease), requiring renal replacement therapy when end-stage renal disease is reached [8].