Nephrol Dial Transplant (2009) 24: 2524–2529
doi: 10.1093/ndt/gfp102
Advance Access publication 4 March 2009

Free and total plasma malondialdehyde in chronic renal insufficiency and in dialysis patients

Amedeo F. De Vecchi[1], Fabrizia Bamonti[2], Cristina Novembrino[3], Silvia Ippolito[2], Luisella Guerra[1], Silvia Lonati[2], Silvia Salini[4], Caroline S. Aman[5], Elisabetta Scurati-Manzoni[2] and Giuliana Cighetti[5]

[1]UO Nephrology and Dialysis, [2]Department of Medical Sciences, University of Milan, [3]Department of Neurological Sciences, University of Milan, 'Dino Ferrari' Center, Fondazione Ospedale Policlinico Mangiagalli e Regina Elena IRCCS, [4]Department of Economics, Business and Statistics and [5]Department of Preclinic Science, LITA Vialba, University of Milan, Milan, Italy

Correspondence and offprint requests to: Giuliana Cighetti; E-mail: giuliana.cighetti@unimi.it

Abstract

Background. Available data about oxidative status in patients with end-stage renal disease (ESRD) or on dialysis are contradictory. The present cross-sectional study aimed to investigate the role of renal insufficiency and dialysis on lipid peroxidation. To separate the effects of uraemia from dialysis-induced stress, we enrolled 26 patients with renal insufficiency on conservative treatment (ESRD), 23 on peritoneal dialysis (PD), 30 on haemodialysis (HD) and 30 controls.

Methods. Plasma malondialdehyde (MDA) levels, both total (tMDA) and free (fMDA), were measured as indexes of oxidative stress by gas chromatography-mass spectrometry. Bound MDA (bMDA) levels were calculated as the difference between tMDA and fMDA.

Results. Total and bMDA concentrations were significantly higher in patients than in controls (ESRD > HD > PD). In PD and HD patients, fMDA levels were similar and significantly higher than in ESRD. Multivariate analysis, with tMDA, fMDA and bMDA as dependent variables, showed similar and significant tMDA and bMDA relations with residual renal function ($t = -2.160$, $P = 0.035$) and albumin ($t = -2.049$, $P = 0.045$). Erythropoietin dose affected only fMDA values ($t = -2.178$, $P = 0.034$).

Conclusions. Free and bMDA concentrations identified different MDA patterns. Bound MDA, not excreted by kidneys, accounts alone for high tMDA concentrations in ESRD patients, while both fMDA and bMDA contribute to tMDA values in dialysis patients. These findings show that increased tMDA could be indicative not only of recent lipid peroxidation, and they also highlight the importance of evaluating free, bound and total MDA in patients with reduced renal function in order to assess their oxidative status.

Keywords: dialysis; gas chromatography-mass spectrometry; lipid peroxidation; malondialdehyde; renal insufficiency

Introduction

Evidence supports the involvement of reactive oxygen species in the pathophysiology of atherosclerotic cardiovascular complications, the most frequent cause of death in renal patients receiving dialysis [1,2]. Oxidative stress, exacerbated by dialysis sessions and by severe chronic inflammation tends, in these patients, to be considered as the cornerstone of the atherosclerotic process [3–10]. Efforts have been made to develop strategies in order to reduce oxidative damage in renal patients on chronic dialysis [11–13]. However, the matter is still controversial since some, but not all authors [14,15], consider peritoneal dialysis (PD) more effective than haemodialysis (HD) in reducing inflammatory and oxidative damage, because of its biocompatibility [5,8].

The occurrence of impaired oxidative status is highlighted by several biomarkers; among them, malondialdehyde (MDA), a terminal compound of lipid peroxidation, is commonly used as an index of oxidative stress [16,17]. In biological matrices, MDA exists both free (fMDA) and bound (bMDA) to SH and/or NH_2 groups of proteins, nucleic acids and lipoproteins [16]. Chemically reactive fMDA is an index of recent and potential damage, while bMDA, excreted by the kidney, is a marker of an older injury [16–18]. In particular, MDA modified LDL can be taken up by tissue macrophages, leading to the formation of foam cells that generate the atherosclerotic plaque [19].

Few and controversial data, often related to differences in assay methodology, are available on MDA concentrations in patients with chronic renal failure. High serum MDA levels were found in patients with advanced chronic renal failure [20,21] and in subjects on PD [5,8], while plasma MDA levels, observed in HD patients, were within the reference interval [12,22]. As reported by other authors, MDA concentrations were significantly higher in HD patients with traditional cardiovascular risk factors than in subjects not at risk [6], and, likewise, higher in patients with serum haemoglobin >10 g/dL than in anaemic subjects [23]. It

Table 1. Demographic, clinical and biochemical characteristics of patients and normal controls

	ESRD ($n = 26$)	PD ($n = 23$)	HD ($n = 30$)	Controls ($n = 30$)
Age (years)	67 ± 16^{a}	60 ± 13	54 ± 13	59 ± 9
Sex (M/F)	20/6	12/11	14/16	18/12
Light smokers	10	12	11	8
Hypertension	25	14	15	–
ACEi or RAAS*	7	6	4	–
Months of dialysis	–	28 ± 27	149 ± 110^{b}	–
EPO dose (UI/week)	2076 ± 3018	1826 ± 3750	$4683 \pm 2774^{b,c}$	–
RRF (mL/min)	$10.1 \pm 3.7^{a,b,d}$	$2.6 \pm 2.5^{a,d}$	0^{d}	95 ± 0.5
tHcy (mmol/L)	35 ± 12^{d}	38 ± 30^{d}	39 ± 12^{d}	9 ± 1
Ferritin (ng/mL)	167 ± 138^{d}	195 ± 177^{d}	132 ± 127^{d}	60 ± 37
Uric acid (mg/dL)	$6.7 \pm 1.6^{a,b,d}$	5.4 ± 1.1^{d}	7.0 ± 1.0^{d}	3.9 ± 1.2
Albumin (g/dL)	4.1 ± 0.4	$3.7 \pm 0.4^{c,d}$	$3.7 \pm 0.3^{c,d}$	4.2 ± 0.8
Haematocrit (%)	34 ± 5^{d}	$30 \pm 3^{c,d}$	$30 \pm 3^{c,d}$	42 ± 3
PTH (pg/mL)	318 ± 174^{d}	229 ± 65^{d}	431 ± 550^{d}	33 ± 15
Cholesterol (mg/dL)	$201 \pm 45^{a,b,e}$	249 ± 60^{d}	171 ± 29^{e}	183 ± 21
HDL cholesterol (mg/dL)	58 ± 19^{a}	$43 \pm 7^{c,d}$	36 ± 13^{d}	61 ± 16
TG (mg/dL)	157 ± 63	174 ± 72	176 ± 78	149 ± 41
CRP (mg/L)**	1.53 ± 4.2	1.55 ± 1.6	0.89 ± 1.4	< 0.5
Vit B_{12} (pmol/L)	398 ± 215	416 ± 161	525 ± 282	480 ± 127

ERSD: patients with renal insufficiency on conservative treatment. PD: patients on peritoneal dialysis. HD: patients on haemodialysis. RRF: residual renal function, EPO: erythropoietin, TG: triglycerides, CRP: C-reactive protein, PTH: parathyroid hormone.
Data are reported as mean ± SD.
*Patients treated with ACEi or RAAS blockers.
**Statistical comparison not performed being all controls' levels below the cutoff value.
[a] $P < 0.001$ versus HD.
[b] $P < 0.001$ versus PD.
[c] $P < 0.001$ versus ESRD.
[d] $P < 0.001$ versus controls.
[e] $P < 0.05$ versus controls.

is important to stress that the analytical procedures, generally adopted for MDA quantification, are based on the reaction with thiobarbituric acid, an assay criticized as not being entirely specific to MDA [16]; this method allows the measurement of only total MDA (tMDA), corresponding to fMDA and bMDA added together. However, the evaluation of both fMDA and tMDA might help understand the actual oxidative status and the '*in vivo*' MDA clearance, respectively.

The present cross-sectional study aimed at investigating the potential effect of renal failure and dialysis techniques on lipid peroxidation. Thus, plasma fMDA and tMDA levels were measured in patients with grade IV–V end-stage renal disease on conservative therapy (ESRD) and in patients on PD or HD. The use of a highly specific reference method based on the gas chromatography–mass spectrometry (GC-MS) technique with isotope dilution [24] enabled us to measure both fMDA and tMDA levels and also to calculate the concentrations of the bMDA. Bound MDA, excreted by the kidney, can be influenced by renal failure and could possibly account for high tMDA concentrations.

Subjects and methods

Patient population

Seventy-nine non-diabetic patients and 30 healthy controls, non-smokers or light smokers, who agreed to participate in the study and fulfilled inclusion criteria, were enrolled in this clinic-based cross-sectional study. The research project was approved by the institutional ethics committee.

Inclusion criteria were as follows: patients with grade IV–V ESRD and stable clinical conditions for 3 months before recruitment were included in the study. All the participants were asked to give an account of their smoking habits, and light smokers (<5 cigarettes/day) were included. ESRD patients undergoing conservative dietary and drug treatment to control clinical and biochemical symptoms of chronic renal failure were identified on the basis of estimated renal creatinine clearance [25] <20 mL/min in two separate instances. PD patients had been on PD for more than 3 months with 3–4 exchanges per day, 2000 mL each, over a 24-h period, adopting a double bag system. HD patients had been on chronic standard HD or acetate-free biofiltration for more than 3 months, 3 times/week for 4 h with bicarbonate as a dialysate buffer, after creatinine clearance had fallen below 8–12 mL/min and/or pharmacological treatment and diet had proved inadequate to control clinical symptoms. HD patients were treated with synthetic or semisynthetic membranes. Vascular access was arteriovenous fistula in the upper limbs.

Exclusion criteria were as follows: ongoing experimental trials, acute infection episodes or peritonitis in the 2 months preceding the study, neoplasm, severe malnutrition or severe hypoalbuminaemia (<3 g/dL), liver cirrhosis, clinically symptomatic cardiac or vascular diseases, heavy smokers (>20 cigarettes/day for a year before recruitment) and age under 20 or over 80 years. Cardiovascular disease was defined on the basis of clinical or radiological evidence of vascular calcification and significant stenosis, electrocardiographic evidence of cardiac ischaemia or atrial fibrillation, history of acute cerebral ischaemia or episodes of 'claudicatio intermittens'. We also excluded PD and HD patients with weekly KT/V values suggesting inadequate dialysis treatment (<1.9 for PD and <3.6 for HD). To have homogeneous groups, we excluded PD patients using amino acid or icodextrin solutions and those treated with automated peritoneal dialysis (APD).

All patients (pre-dialysis, PD patients and those on standard bicarbonate HD dialysis in the morning sessions), who met these criteria, were asked to participate in the study and sign informed consent. Twenty-six ESRD, 23 PD and 30 HD patients agreed to take part in the study. Demographic, clinical and biochemical characteristics of these subjects are reported in Table 1. Arterial blood pressure higher than 140/80 on three separate occasions without any antihypertensive therapies was defined as hypertension.

All dialysis and ESRD anaemic patients were given adequate doses of alpha or beta erythropoietin (EPO) when necessary, and oral folate supplementation in order to maintain serum haemoglobin between 11 and

Table 2. Total, free and bound malondialdehyde levels in dialysis, end-stage renal disease patients and controls

	ESRD ($n = 26$)	PD ($n = 23$)	HD ($n = 30$)	Controls ($n = 30$)
tMDA (μmol/L)	$6.95 \pm 1.5^{a,b,d}$	4.40 ± 1.1^{d}	5.70 ± 1.3^{d}	1.70 ± 0.4
fMDA (μmol/L)	0.47 ± 0.3	$1.20 \pm 0.6^{c,d}$	$1.30 \pm 0.6^{c,d}$	0.45 ± 0.1
bMDA (μmol/L)	$6.48 \pm 1.7^{a,b,d}$	3.20 ± 1.0^{d}	$4.40 \pm 1.2^{b,d}$	1.25 ± 0.3

Bound MDA level was calculated as the difference between total and free measured MDA levels. Data are reported as mean ± SD.
[a]$P < 0.001$ versus HD.
[b]$P < 0.001$ versus PD.
[c]$P < 0.001$ versus ESRD.
[d]$P < 0.001$ versus controls.

12 g/dL and serum folate above 7 nmol/L. As 14 ESRD, 21 PD and 25 HD patients were given 5–30 mg/week folate supplementation, final serum folate concentrations, variable within and between groups were not reported.

Methods

Peripheral venous blood samples were drawn from controls, ESRD and PD patients after an overnight fast, and from HD patients after the longest interval between two dialysis sessions. Plasma and serum were divided into aliquots that were immediately used or stored at −80°C according to the analytical regime.

In PD patients with a daily urine output of >100 mL, residual renal function (RRF) was calculated as the mean of creatinine and urea renal clearances was measured on 24-h urine output, without suspending PD.

Plasma total homocysteine (tHcy) and serum-related vitamins (B_{12} and folate) were determined by the relevant commercial kits on AxSYM analyser (Abbott Diagnostics, Abbott Park, IL, USA). Haematocrit was determined by routine counter (STKS, Beckman Coulter, Miami, FL, USA). Uric acid, ferritin, albumin, total and HDL cholesterol, parathyroid hormone (PTH), triglycerides (TG) and C-reactive protein (CRP) were assessed by standard laboratory techniques.

Free MDA and tMDA concentrations were determined on plasma samples by the GC-MS technique using synthesized dideuterated MDA (d_2-MDA) as internal standard added to the biological samples before any analytical manipulation [24]. Briefly, plasma (0.2 mL) was diluted with a 0.2 mL citric buffer (0.4 mol/L; pH 4.0), added with butylated hydroxytoluene (0.5 mmol/L; 5 nmol) and d_2-MDA (0.25 nmol) to determine free MDA. Samples, derivatized with phenylhydrazine at room temperature for 30 min, were extracted with hexane and analysed by the GC-MS method [24]. The same procedure was used to evaluate the plasma tMDA concentration. However, before derivatization, the samples were submitted to hydrolysis in 1 mol/L NaOH at 60°C for 60 min [24]. The bMDA level was calculated as the difference between total and fMDA.

Statistical analysis

Data are reported as mean ± standard deviation (SD). On the basis of the Kolmogorov–Smirnov and Shapiro–Wilk normality test, the values of all the response variables were normally distributed; among the explanatory variables, only EPO dose and homocysteine levels were not normally distributed. The normal Q–Q plot of residuals also supported the assumption of normality of residuals. Statistical comparison was made by Student's *t*-test for unpaired data. A multiple regression analysis was performed by the ordinary least-square method in order to measure the influence of patients' demographic, clinical and biochemical characteristics on MDA levels. Specifically, in three different models with dependent variables fMDA, tMDA and bMDA concentrations, respectively, the MDA levels were regressed for age, homocysteine, albumin, uric acid, serum ferritin, haematocrit, total and HDL cholesterol, TG, CRP, PTH, EPO dose, RRF concentrations and dialysis duration. All explanatory variables were entered in block. Variables were considered statistically significant when $P < 0.05$. All statistical calculations were made by the SPSS 12.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

As reported in Table 1, dialysis duration was longer, and EPO dose was higher in HD than in PD patients. ESRD patients had significantly higher RRF than those on dialysis. Twenty PD patients (87%) had RRF ranging from 0.13 to 8.50 mL/min (mean 3.26 ± 2.36), whereas HD patients' residual urine output was not >100 mL/24 h in 3 consecutive days. Therefore, residual renal clearance was almost absent in HD patients. Mean values of tHcy, uric acid and ferritin levels were higher in all patient groups than in controls. Uric acid concentrations were also significantly higher in ESRD and HD than in PD patients. Both PD and HD patients had lower serum albumin and haematocrit than ESRD and controls. Plasma total cholesterol levels were significantly higher in PD patients than in HD and ESRD, and higher in ESRD than in HD. HD patients had the lowest HDL cholesterol levels, followed by PD and ESRD patients.

No statistically significant differences among the groups were observed in vitamin B_{12}, TG and PTH levels, with the last parameter significantly higher in the three groups of patients compared to controls.

Plasma tMDA concentrations were significantly higher in each group of patients than in controls (Table 2), and the highest levels were observed in ESRD followed by HD and PD patients. Free MDA levels, similar in ESRD and controls, were 3-fold higher in both dialysis groups. Bound MDA values paralleled those of tMDA.

Three models of multivariate analysis were performed considering tMDA, fMDA and bMDA, each as response variables, and the type of clinical treatment together with all the other detected indexes as explanatory variable. Details of significant fMDA and tMDA relationships are reported in Table 3. A significant negative correlation was found between fMDA and EPO doses ($t = -2.178$, $P = 0.034$). A similar significant negative relationship was also found between tMDA and both albumin levels ($t = -2.049$, $P = 0.045$) and RRF ($t = -2.160$, $P = 0.035$) and between bMDA and both albumin levels ($t = -2.120$, $P = 0.039$) and RRF ($t = -2.270$, $P = 0.027$) when calculated bMDA was considered as a dependent variable. No effect of dialysis duration on MDA concentrations was observed in either PD or HD. No relationships were observed between MDA values and age or other considered biochemical findings.

Table 3. Multiple regression analysis

	fMDA (μmol/L) dependent variable				tMDA (μmol/L) dependent variable			
	Regression coefficient	SE	*t*	*P*-value	Regression coefficient	SE	*t*	*P*-value
ESRD[a]	−0.027	1.003	−0.127	0.900	9.255	2.341	3.953	0.000
HD	1.114	0.208	5.346	0.000	−1.058	0.486	−2.176	0.034
PD	0.818	0.257	3.183	0.002	−1.709	0.600	−2.849	0.006
Albumin[b]	−0.029	0.171	−0.169	0.866	−0.818	0.399	−2.049	0.045
EPO dose[c]	< −0.001	0.001	−2.178	0.034	<0.001	0.001	−1.502	0.139
RRF[d]	< −0.001	0.001	−0.094	0.926	−0.002	0.001	−2.160	0.035

Only the significant variables are summarized in the table.
The significant bMDA relationships are listed in the text.
[a]Intercept.
[b]g/dL.
[c]UI/week.
[d]mL/min.

Discussion

The present study was made to investigate the role of renal insufficiency and dialysis technique on lipid peroxidation, and to separate the effects of the uraemic environment from dialysis-induced oxidative stress. We selected patients with ESRD just before dialysis, and patients on peritoneal or HD. An additional original feature of this study was the measurement of both plasma tMDA, an index of total injury (bound + free), and fMDA, an index of recent or potential damage [16]. The measurement of both tMDA and fMDA concentrations allowed us to calculate bMDA amounts and to study the oxidative status in these patients.

In our study, tMDA concentrations were significantly higher in ESRD and dialysis patients than in controls, confirming the presence of oxidative stress both in ESRD and in dialysis patients [3–6,12,20–23]. These results agree with the high tMDA levels observed by other authors in HD patients [3,10,23,26,27] but contrast sharply with reported normal tMDA values in these patients [12,22].

If we had only measured tMDA concentrations, as usually done by the reaction with thiobarbituric acid, we would have simply concluded that lipid peroxidation is more prominent in ESRD, thereby supporting the hypothesis that it is renal failure, rather than dialysis that induces lipid peroxidation [9,26–29]. From this point of view, the peritoneal technique appears to be more effective than HD [5,8]. However, apparently contrasting results were obtained from fMDA evaluation. In fact, fMDA concentrations similar in ESRD and in controls, but significantly higher in both PD and HD patients, confirm the occurrence of lipid peroxidation in dialysis patients but not in kidney failure, against the claim that lipid peroxidation is a mere consequence of uraemic environment [9,26–29] and in contrast to increased tMDA levels reported in these patients by us and by other authors [20,21]. It is important to note that in ESRD, bMDA accounts almost alone for high tMDA levels, whereas in dialysis patients high tMDA is due to both free and bMDA.

The three forms of MDA have never been evaluated together in uraemic patients. Ours is the first study to deal with and to discuss the behaviour of both fMDA and tMDA in ESRD and dialysis patients. Plasma fMDA levels depend on the balance between MDA formation and its detoxification as bMDA excreted by urine after its transformation from protein MDA adducts to simpler MDA adducts by proteolytic enzymes. Thus, tMDA represents the sum of bMDA (that has not been cleared by either native kidneys or dialysis or has not been enzymatically transformed into simple MDA adducts) and fMDA. Therefore, tMDA itself cannot reveal whether increased levels in MDA account for increased lipid peroxidation and/or increased levels in MDA binding to proteins and/or decreased bMDA renal clearance. Among plasma proteins, albumin is considered a major oxidation target in uraemia with many thiol-free groups [30] that can undergo oxidative modifications by oxidation and/or by reacting with different carbonyl compounds like MDA.

In our study, bMDA levels, higher in HD than in PD patients, might result from the lack of urinary excretion or from inadequate removal by HD and/or enzymatic transformation of protein-bound MDA adducts into simpler adducts before excretion in urine [17]. In fact, a complete loss of RRF is generally observed in HD and not in most of PD patients. In addition, we should expect a larger and continuous bMDA clearance through the peritoneal membrane due to increased protein clearance associated with PD. Moreover, increased bMDA concentrations, observed in our HD patients, might be consistent with elevated MDA-lysine adduct levels, detected by some authors [29] in the plasma of both diabetic and non-diabetic HD patients after acidic proteins hydrolysis, and may be due to the fact that over 90% of the MDA-lysine adducts are bound to albumin, as demonstrated by other authors [15]. It is more difficult to understand why bMDA levels are higher in ESRD with RRF than in patients with no renal function at all like HD patients. This can only be explained by assuming that dialysis leads to higher bMDA clearance than excretion from severely diseased kidneys with few functioning nephrons. Moreover, ESRD patients' biochemical conditions (e.g. normal albumin concentration), unlike those of dialysis patients, might help increase bMDA while maintaining low fMDA levels.

The significant negative relations resulting from multivariate statistical analysis confirm our hypothesis on the role of both RRF and albumin on plasma total and

bound MDA levels; specifically, the negative relationship between total and bMDA and albumin might be explained by considering that the albumin–thiol groups can be oxidatively modified not only by reacting with MDA. The negative relationship between albumin and F_2-isoprostanes observed in HD patients [31] and the positive correlation between MDA and F_2-isoprostanes reported by others [31,32] corroborate our negative correlation between the two MDA forms and albumin.

The lipid concentration and CRP covariates can both affect lipid peroxidation increasing MDA levels. In our patients, no relation between MDA forms and triglyceride, HDL and total cholesterol was found. The lack of relation between MDA and CRP might be due to the CRP low mean values observed in our patients, partly because of the exclusion of infectious, malnourished and neoplastic patients.

Unlike other authors, we did not find any significant relationship between dialysis duration and values of MDA forms [10]. Some controversial data have been reported on the removal of tMDA by the HD technique; the majority of authors observed a rapid decrease in tMDA after HD sessions [3,7,33], while others did not [27]. The significant decrease in plasma tMDA observed by some authors [33] after HD sessions is thought to be due to MDA low molecular weight and water solubility. However, theoretically, HD is likely to clear only the small free MDA molecules (23% of tMDA in our HD patients), but not the MDA bound to peptides, albumin and lipoproteins, which form high molecular weight compounds. We did not collect post-dialysis samples, in order to avoid the heparin-mediated effect and the activation of a coagulation pathway triggered by the contact with dialysis membranes. We have, therefore, no data on which MDA form is more easily cleared by HD. To the best of our knowledge, no data have been published on tMDA removal by PD session.

Multivariate analysis also shows the decisive effect of EPO doses on the reduction of fMDA concentrations that might be caused by a prooxidant effect of EPO, as reported by some [34] but not confirmed by all authors [23,35,36]. On the other hand, it might also be related to the severity of anaemia before EPO treatment. At present, we cannot therefore discriminate between a prooxidant effect of EPO *per se* and hyperoxidation caused by anaemia or by factors reducing EPO pharmacological activity (infection, inflammation, malnutrition, hyperparathyroidism, etc). Moreover, albumin and RRF possibly weaken the correlation between factors. In particular, the regression model, including RRF with zero value in all HD patients, might challenge the homoscedasticity assumptions of multivariate analysis.

In conclusion, our results confirm the presence of lipid peroxidation in ESRD, HD and PD patients. The detection of both free and tMDA concentrations helps to explain the different MDA patterns in patients with renal insufficiency. In ESRD patients, high tMDA levels are due only to bMDA levels not cleared by the diseased kidneys, whereas high tMDA levels in dialysis patients are due to both free and bMDA concentrations. Finally, our study highlights the importance of evaluating the three forms of MDA in order to understand the role of oxidative stress, especially in patients with reduced renal function. In fact, the increase in tMDA concentrations appears to be not only due to recent lipid peroxidation, but also to bMDA not removed by kidneys or by dialysis.

Acknowledgements. This study was supported partly by grants to F.B. and G.C from Italian Ministry of Research. The authors are very grateful to Mrs Mary Coduri for her linguistic consultation.

Conflict of interest statement. None declared.

References

1. Himmelfarb J, Stenvinkel P, Ikizler TA *et al*. The elephant in uremia: oxidative stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62: 1524–1538
2. Locatelli F, Canaud B, Eckardt KU *et al*. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol Dial Transplant* 2003; 18: 1272–1280
3. Jackson P, Loughrey CM, Lightbody JH *et al*. Effect of hemodialysis on total antioxidant capacity and serum antioxidants in patients with chronic renal failure. *Clin Chem* 1995; 41: 1135–1138
4. Fiorillo C, Oliviero C, Rizzuti G *et al*. Oxidative stress and antioxidant defenses in renal patients receiving regular haemodialysis. *Clin Chem Lab Med* 1998; 36: 149–153
5. Martin-Mateo MC, del Canto-Jafiez E, Barrero-Martinez MJ. Oxidative stress and enzyme activity in ambulatory renal patients undergoing continuous peritoneal dialysis. *Ren Fail* 1998; 20: 117–124
6. Boaz M, Matas Z, Biro A *et al*. Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int* 1999; 56: 1078–1083
7. Gerardi GM, Usberti M, Martini G *et al*. Plasma total antioxidant capacity in hemodialyzed patients and its relationships to other biomarkers of oxidative stress and lipid peroxidation. *Clin Chem Lab Med* 2002; 40: 104–110
8. Samouilidou EC, Grapsa EJ, Kakavas I *et al*. Oxidative stress markers and C-reactive protein in end-stage renal failure patients on dialysis. *Int Urol Nephrol* 2003; 35: 393–397
9. Oberg BP, McMenamin E, Lucas FL *et al*. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004; 65: 1009–1016
10. Koken T, Serteser M, Kahraman A *et al*. Changes in serum markers of oxidative stress with varying periods of haemodialysis. *Nephrology* 2004; 9: 77–82
11. Clermont G, Lecour S, Cabanne JF *et al*. Vitamin E-coated dialyzer reduces oxidative stress in hemodialysis patients. *Free Radic Biol Med* 2001; 31: 233–241
12. Mydlik M, Derzsiova K, Racz O *et al*. A modified dialyzer with vitamin E and antioxidant defense parameters. *Kidney Int* 2001; 59: S144–S147
13. Sosa MA, Balk EM, Lau J *et al*. A systematic review of the effect of the Excebrane dialyser on biomarkers of lipid peroxidation. *Nephrol Dial Transplant* 2006; 21: 2825–2833
14. Krediet RT. The peritoneal membrane in chronic peritoneal dialysis. *Kidney Int* 1999; 55: 341–356
15. Miyata T, Horie K, Ueda Y *et al*. Advanced glycation and lipidoxidation of the peritoneal membrane: respective roles of serum and peritoneal fluid reactive carbonyl compounds. *Kidney Int* 2000; 58: 425–435
16. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radic Biol Med* 1991; 11: 81–128
17. Draper HH, Csallany AS, Hadley M. Urinary aldehydes as indicators of lipid peroxidation *in vivo*. *Free Radic Biol Med* 2000; 29: 1071–1077
18. Draper HH, Hadley M, Lissemore L *et al*. Identification of N-ε-(2-propenal) lysine as a major urinary metabolite of malondialdehyde. *Lipids* 1988; 23: 626–628

19. Holvoet P, Vanhaecke J, Janssens S *et al*. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998; 98: 1487–1494
20. Zwolinska D, Grzeszczak W, Kilis-Pstrusinska K *et al*. Lipid peroxidation and antioxidant enzymes in children with chronic renal failure. *Pediatr Nephrol* 2004; 19: 888–892
21. Rutkowski P, Malgorzewicz S, Slominska E *et al*. Interrelationship between uremic toxicity and oxidative stress. *J Ren Nutr* 2006; 16: 190–193
22. Erdogan C, Unlucerci Y, Turkmen A *et al*. The evaluation of oxidative stress in patients with chronic renal failure. *Clin Chim Acta* 2002; 322: 157–161
23. Sommerburg O, Grune T, Hampl H *et al*. Does treatment of renal anemia with recombinant erythropoietin influence oxidative stress in hemodialysis patients? *Clin Nephrol* 2000; 53: S23–S29
24. Cighetti G, Debiasi S, Paroni R *et al*. Free and total malondialdehyde assessment in biological matrices by gas chromatography-mass spectrometry: what is needed for an accurate detection. *Anal Biochem* 1999; 266: 222–229
25. Cockroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41
26. Hirayama A, Nagase S, Gotoh M *et al*. Hemodialysis does not influence the peroxidative state already present in uremia. *Nephron* 2000; 86: 436–440
27. Trimarchi H, Mongitore MR, Baglioni P *et al*. N-acetylcysteine reduces malondialdehyde levels in chronic hemodialysis patients: a pilot study. *Clin Nephrol* 2003; 59: 441–446
28. Terawaki H, Yoshimura K, Hasegawa T *et al*. Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin. *Kidney Int* 2004; 66: 1988–1993
29. Miyata T, Fu MX, Kurokawa K *et al*. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 1998; 54: 1290–1295
30. Himmelfarb J, McMonagle E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int* 2000; 58: 2571–2578
31. Lim P-S, Chang Y-M, Thien L-M *et al*. 8-Iso-prostaglandin $F_{2\alpha}$ as a useful clinical biomarker of oxidative stress in ESRD patients. *Blood Purif* 2002; 20: 537–542
32. Cavalca V, Sisillo E, Veglia F *et al*. Isoprostanes and oxidative stress in off-pump and on-pump coronary bypass surgery. *Ann Thorac Surg* 2006; 81: 562–567
33. Lucchi L, Iannone A, Bergamini S *et al*. Comparison between hydroperoxides and malondialdehyde as markers of acute oxidative injury during hemodialysis. *Artif Organs* 2005; 29: 832–837
34. Nemeth I, Turi S, Hazon I *et al*. Vitamin E alleviates the oxidative stress of erythropoietin in uremic children on hemodialysis. *Pediatr Nephrol* 2000; 14: 13–17
35. Ludat K, Sommerburg O, Grune T *et al*. Oxidation parameters in complete correction of renal anemia. *Clin Nephrol* 2000; 53: S30–S35
36. Marsillach J, Martinez-Vea A, Marcas L *et al*. Administration of exogenous erythropoietin β affects lipid peroxidation and serum paraoxonase-1 activity and concentration in predialysis patients with chronic renal disease and anaemia. *Clin Exp Pharmacol Physiol* 2007; 34: 347–349

Received for publication: 18.6.08; Accepted in revised form: 19.2.09

Nephrol Dial Transplant (2009) 24: 2529–2536
doi: 10.1093/ndt/gfp104
Advance Access publication 16 March 2009

Sudden death and associated factors in a historical cohort of chronic haemodialysis patients

Simonetta Genovesi[1,2], Maria G. Valsecchi[3], Emanuela Rossi[3], Daniela Pogliani[1], Irene Acquistapace[1], Vincenzo De Cristofaro[4], Andrea Stella[1,2] and Antonio Vincenti[5]

[1]Department of Clinical Medicine and Prevention, University of Milano-Bicocca, [2]Nephrology Unit, San Gerardo Hospital, Monza, [3]Department of Clinical Medicine and Prevention, Medical Statistic Unit, University of Milano-Bicocca, Milano, [4]Nephrology Unit, Sondrio Hospital, Sondrio and [5]Electrophysiology and Cardiac Pacing Unit, San Gerardo Hospital, Monza, Italy

Correspondence and offprint requests to: Simonetta Genovesi; E-mail: simonetta.genovesi@unimib.it

Abstract

Background. In haemodialysis patients, deaths due to cardiovascular causes constitute a large proportion of total mortality and sudden cardiac deaths account for ∼22% of all deaths. The aim of this study was to evaluate the incidence of sudden cardiac death and associated risk factors in a cohort of haemodialysis patients.

Methods and results. The 3-year cumulative incidence of death in a cohort of 476 patients on chronic haemodialysis treatment was 34.3% (SE 2.3). Sudden death had a 6.9% (SE 1.2) cumulative incidence, with 32 events representing 19.2% of all deaths, while cardiovascular not sudden death and noncardiovascular death accounted for a 3-year cumulative incidence of 7.3% (SE 1.2) and 20.1% (SE 1.9), respectively. According to Cox multivariate analysis, significant risk factors for sudden death were the presence of atrial fibrillation, diabetes mellitus, predialytic hyperkalaemia, haemodialysis mode and C-reactive protein level, which were associated with a 2.9 ($CI_{95\%}$ 1.3–6.4), 3.0 ($CI_{95\%}$ 1.3–7.2), 2.7 ($CI_{95\%}$ 1.3–5.8), 4.5 ($CI_{95\%}$ 1.3–15.5) and 3.3 ($CI_{95\%}$ 1.2–8.8)-fold increase in the risk of sudden death, respectively. Sudden death was significantly more