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Genetic damage in patients with chronic kidney disease, peritoneal dialysis and haemodialysis: a comparative study

Angélica Rangel-López[1,*], Maria Eugenia Paniagua-Medina[1], Marcia Urbán-Reyes[2], Martha Cortes-Arredondo[3], Cleto Álvarez-Aguilar[3], Joel López-Meza[4], Alejandra Ochoa-Zarzosa[4], Bengt Lindholm[5], Elvia García-López[5] and José Ramón Paniagua[1]

[1]Medical Research Unit on Nephrological Diseases, Specialties Hospital, National Medical Centre, Century XXI, Mexican Institute for Social Security (IMSS), Mexico City, Mexico, [2]Gastroenterology and Nutrition Department, Infantile Hospital 'Federico Gomez', Ministry of Health, Mexico City, Mexico, [3]Medical Research Unit on Clinical Epidemiology, Regional General Hospital No. 1, Mexican Institute for Social Security (IMSS), Morelia, Mexico, [4]Multidisciplinary Center for Studies on Biotechnology, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico and [5]Division of Renal Medicine and Baxter Novum, Karolinska Institute, Stockholm, Sweden

*To whom correspondence should be addressed. Unidad de Investigación Médica en Enfermedades Nefrológicas, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Cuauhtémoc 330, Col. Mexico, D.F. 06725, Mexico. Tel: +52-55-56276900 (Ext: 21371); Fax: +52-55-57610952; Email: ragn62@prodigy.net.mx

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Patients with chronic kidney disease (CKD) have signs of genomic instability and, as a consequence, extensive genetic damage, possibly due to accumulation of uraemic toxins, oxidative stress mediators and other endogenous substances with genotoxic properties. We explored factors associated with the presence and background levels of genetic damage in CKD. A cross-sectional study was performed in 91 CKD patients including pre-dialysis (CKD patients; *n* = 23) and patients undergoing peritoneal dialysis (PD; *n* = 33) or haemodialysis (HD; *n* = 35) and with 61 healthy subjects, divided into two subgroups with the older group being in the age range of the patients, serving as controls. Alkaline comet assay and cytokinesis-block micronucleus assay in peripheral blood lymphocytes were used to determine DNA and chromosome damage, respectively, present in CKD. Markers of oxidative stress [malondialdehyde (MDA), advanced glycation end products (AGEs), thiols, advanced oxidation protein products and 8-hydroxy-2′-deoxyguanosine] and markers of inflammation (C-reactive protein, interleukin-6 and tumour necrosis factor alpha) were also measured. Micronucleus (MN) frequency was significantly higher ($P < 0.05$) in the CKD group (46±4‰) when compared with the older control (oC) group (27.7±14). A significant increase in MN frequency ($P < 0.05$) was also seen in PD patients (41.9±14‰) versus the oC group. There was no statistically significant difference for the HD group (29.7±15.6‰; *P* = NS) versus the oC group. Comet assay data showed a significant increase ($P < 0.001$) of tail DNA intensity in cells of patients with CKD (15.6±7%) with respect to the total control (TC) group (11±1%). PD patients (14.8±7%) also have a significant increase ($P < 0.001$) versus the TC group. Again, there was no statistically significant difference for the HD group (12.5±3%) compared with the TC group. Patients with MN values in the upper quartile had increased cholesterol, triglycerides, AGEs and MDA levels and lower albumin levels. Multiple logistic regression analysis showed that male gender, diabetes and treatment modality were independently associated with higher levels of DNA damage. Our results suggest that oxidative stress, diabetes, gender and dialysis modality in CKD patients increased DNA and chromosome damage. To confirm these data, prospective clinical trials need to be performed.

Introduction

Chronic kidney disease (CKD) is a progressive condition marked by deteriorating kidney function over time. The early stages of CKD (stages 2 and 3) are manifested by kidney damage and are generally asymptomatic, whereas more advanced stages of CKD (stages 4 and 5) require treatment of uraemia and its complications and ultimately renal replacement therapy when end-stage renal disease (ESRD) is reached (1). Patients with CKD have signs of extensive DNA damage and an elevated risk for developing cancer (2–4). This risk may be related to impairment of DNA repair. DNA lesions may induce mutations in oncogenes and tumour-suppressor genes that may lead to malignancies if mutagenicity is not mitigated by repair mechanisms. Elevated frequencies of micronuclei (MN) in cultured peripheral blood lymphocytes (PBL) of patients before and after renal therapy have been found (5). MN formation has been validated to be a biomarker for environmental mutagen and carcinogen exposures and genomic instability and is extensively used as an indicator of chromosomal damage and early biomarker of cancer risk in non-uraemic population (6).

In CKD patients, impairment of DNA damage repair and increased chromosome damage may be caused by the uraemic state as well as by chronic inflammation linked to increased formation of reactive oxygen species (7,8). Even though a negative impact of reactive oxygen species and reactive nitrogen species (collectively denoted RONS) in CKD has not been clearly demonstrated, increased RONS lead to DNA strand breaks, point mutations and aberrant DNA cross-linking, thereby causing genomic instability (9,10). RONS have been postulated to be an important risk factor for cardiovascular disorders and the development of cancer (11) and could be responsible for genomic instability in CKD patients. Furthermore, advanced glycation end products (AGEs) could play a role in causing DNA damage (3,12,13). In addition to the biological implications of renal disease and uraemic milieu on the integrity of DNA, the modality and duration of the uraemia treatment may also influence the degree of DNA damage or the mechanisms of repair (4,8,14). DNA integrity has been analysed through two methodologies, the cytokinesis-block micronucleus (CBMN) assay (15,16) and the comet assay (17). The CBMN assay detects chromosome breakage, chromosome

loss and chromosome rearrangement in cells that completed nuclear division once (15,16). The comet assay is an accurate method for measuring DNA oxidation in individual cells, and it is a standard technique for evaluating DNA damage and/or repair due to its sensitivity for detecting low levels of DNA damage and might be a useful tool to assess DNA damage in PBL of CKD patients. Another methodology is measurement of the 8-hydroxy-2′-deoxyguanosine (8-OHdG) content in leukocyte DNA (18). The level of 8-OHdG, a prevalent pro-mutagenic oxidation product of guanine, reflects nuclear damage due to small oxidative changes and can give rise to G-to-T transversion mutations in key genes known to be involved in the development of cancer (18,19); however, direct evidence of a cause-and-effect relation between DNA oxidation and carcinogenesis has not yet been reported. DNA integrity may be associated with different clinical and biochemical factors, but few previous studies have simultaneously analysed correlations between clinical variables, biochemical factors, dialysis treatment and DNA damage detected by either the comet assay or the CBMN frequency. In this context, the genetic damage can be defined as the presence of multiple genetic alterations (errors in replication, errors in mitosis, carcinogens, DNA adducts, alkali-labile sites, strand breaks, etc.), both at the level of DNA sequence and cytogenetic, and alterations in the cell cycle checkpoints (20,21). Biomonitoring of genetic damage has been carried out in PBL by measurement of the MN frequency and the comet assay, and several biological parameters of ESRD patients have been measured to determine their burden of this DNA damage. Structural DNA damage, such as single- and double-strand breaks and alkali-labile sites, detectable with the comet assay, was significantly increased in lymphocytes of patients under dialysis (17,22). These types of DNA damage are theoretically repairable. Yet, DNA repair capacity is reduced by prolonged haemodialysis (HD) therapy (23). As mentioned above, unrepaired or improperly repaired DNA lesions may have serious consequences, such as premature ageing (24,25), atherosclerosis (26) and cancer predisposition (27). Furthermore, sister chromatid exchanges (which indicate repair on damaged sites) and chromosome breaks have been found in lymphocytes of dialysis patients (5,28,29).

As the comet assay measures primary DNA damage, this must be repaired, in contrast to what occurs with fixed damage. The CBMN assay is a very important cytogenetic test because of its feasibility and ability to detect clastogenic and aneugenic effects. The MN are DNA-containing particles that arise during mitosis and result from unrepaired DNA double-strand breaks, leading to chromatin fragments or whole chromosomes incorrectly distributed during mitosis. Several studies have reported increased MN frequencies in ESRD (4,5,29).

The aim of this study was to investigate the degree of DNA damage and its relationship with markers of oxidative stress and inflammation in CKD patients undergoing conservative treatment, peritoneal dialysis (PD) or HD therapy.

Methods

Subjects

In a cross-sectional study, the background levels of DNA effects in PBL were evaluated by the CBMN assay and the comet assay conducted in CKD patients and ESRD patients undergoing dialysis treatment. The protocol was approved by the Ethics and Scientific Research Committees of the participating hospitals. Patients and controls signed a written informed consent letter before enrolment. A total of 91 adult CKD patients including 23 CKD stage 4 non-dialysed (CKD4-ND) patients and 68 CKD stage 5 patients undergoing either PD (n = 33) or HD (n = 35) were enrolled from outpatient CKD clinics and dialysis clinics at the participating hospitals. The CKD4-ND patients who had an estimated glomerular filtration rate (eGFR) of 22.5 ml/min received conventional treatment. Patients on PD received standard treatment with four exchanges of 2 l of glucose-based solutions daily. HD patients received dialysis 4 h or more, three times per week, with polysulphonic membrane. All patients received erythropoietin and calcium-based phosphate binders as appropriate. Patients with oncological diseases, bacterial infections, seropositivity for hepatitis C or B or HIV, hepatic insufficiency or immunosuppressive therapy were excluded. Healthy volunteers (n = 61) who met the inclusion criteria served as control subjects. Clinical records from all subjects were reviewed, and the relevant data recorded.

Sample collection

After a 12-h fast and a 20-min rest, samples were taken from all patients between 8 and 9 AM (in HD patients, in the midday, midweek session) from the antecubital vein; venous blood was drawn into plastic tubes containing ethylenediaminetetraacetic acid (EDTA), heparin and tubes without any addition (BD-Vacutainer, Plymouth, UK). Blood samples were protected from light using aluminium foil and centrifuged immediately. Aliquots were prepared for the determination of routine clinical chemistry variables using standard auto-analyser techniques (Clinical-Chemistry-System, AR-Modular-IIP, Roche-Diagnostics, Switzerland). The remaining aliquots were stored at –80°C until they were analysed.

Inflammation and oxidative stress markers

Serum concentrations of interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) were determined by enzyme-linked immunosorbent assay (ELISA; Assay-Designs®), according to the manufacturer's instructions. High sensitivity C-reactive protein (hs-CRP) was measured by nephelometry (Dade-Behring, Switzerland). Oxidative stress tests, malondialdehyde (MDA), AGEs-associated fluorescence, thiols and advanced oxidation protein products (AOPP), were determined according to routine procedures by Research Laboratory at the National Medical Center (IMSS). Serum 8-OHdG was measured using a commercially available competitive ELISA kit (Enzo® Life Sciences, Plymouth, USA) by diluting the samples. The kit can measure 8-OHdG values ranging from 0.94 to 60 ng/ml, using a monoclonal specific antibody. This antibody does not cross-react with the original four deoxyribonucleosides, 29-deoxyinosine, 8-hydroxy-27-deoxyadenine or O6-methyl-29-deoxyguanosine.

DNA and chromosome damage markers

CBMN assay (16) and comet assay performed under alkaline condition (30) in PBL were used to determine DNA and chromosome damage. Mononuclear cells were isolated with the use of a Ficoll density gradient (Ficoll-paque PLUS, Amersham, Biosciences, USA), according to standard protocols. PBL were resuspended in RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum (hiFCS), 10% dimethylsulfoxide (DMSO) and cooled slowly to –80°C.

Cytokinesis-block micronucleus assay. The CBMN assay was performed according to Fenech (16) with slight modifications. Briefly, lymphocyte cultures were set up in tubes by adding whole blood to PB-MAX-karyotyping medium (Gibco, Invitrogen™, USA) containing RPMI-1640 medium, 15% hiFCS, 1% antibiotics, L-glutamine and 1% phytohaemagglutinin, and lymphocytes were incubated for 72 h at 37°C with 5% CO_2. Two cultures per subject were established. Cytochalasin-B (6 µg/ml) was added to the cultures 44 h later. At 72-h incubation, the cultures were harvested by centrifugation (800 rpm/8 min) and treated with a hypotonic solution (0.075 M KCl/4°C). The cells were then centrifuged and a methanol solution was added three times, and the resulting cells were resuspended and dropped onto clean slides. The slides were stained with Wright/10% Giemsa in phosphate buffer (pH 6.8) and scored. A total of 1000 binucleated cells (BNC) with well-preserved cytoplasm and nuclei (500 per replicate) were scored per subject on coded slides and the frequency of MN/1000 BNC was determined. To avoid differences between observers, the same individual carried out all the microscopic analyses. MN was scored according to the standard recognition criteria (http://www.humn.org) (31).

Comet assay. The alkaline version of the comet assay was performed according to the method of Singh *et al.* (30) with slight modifications. In brief, 25 µl of the single suspension of the cells of interest are suspended in low melting agarose (LMA) 0.5%, in calcium- and magnesium-free phosphate-buffered saline (fCa/Mg-PBS buffer) and layered onto fully frosted microscope slides pre-coated with 480 µl of standard agarose (SA) 0.75% in fCa/Mg-PBS buffer. A final layer of 100 µl of 0.5% LMA was added on top. Slides were immersed in a jar containing cold lysing solution (1% Triton X-100; 10% DMSO; 10 mM Tris; 2.5 M NaCl; 1 mM Na_2EDTA with pH 10 at –4°C for 1 h). Slides were pretreated for 20 min in unwinding buffer (300 mM NaOH; 1 mM Na_2EDTA/pH 13). Electrophoresis was carried out using the same solution buffer for 20 min/25 V and 300 mA (0.8 V/cm). Pre-incubation and electrophoresis were

performed in an ice bath. Afterwards, the slides were washed three times in 0.4 M Tris/pH 7.4, and DNA was stained by adding 20 µl of ethidium bromide (10 µg/ml). Tail parameters were then calculated using image analysis systems fitted with an Olympus BX50 fluorescence microscope that was equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. Images of at least 100 randomly selected cells (50 cells from each of two slides) were analysed from each individual using the software program, Image-ProPlus version 4.0. Tail parameter used in this study was tail DNA intensity, which covers the widest range of damage and is linearly related to break frequency over most of this range. The percentage of DNA in the tail region and in head of the comet is thought to be directly proportional to DNA damage.

Statistical analysis

Data are presented as mean ± SD when continuous variables or frequencies for discontinuous variables are reported. Differences between groups were analysed by Student's *t*-test or chi-square test, according to the characteristics of variables. Correlations were analysed with Pearson coefficient and a logistic regression analysis was used to detect variables independently associated with increased level of DNA damage. A P value <0.05 was considered significant. All calculations were performed using SPSS v14 software for Windows (SPSS Inc., Chicago, IL, USA).

Results

The demographic characteristics of the study population

Table I summarises the demographics, clinical and biochemical data for the different groups of patients and controls. Data are presented as mean ± SD when continuous variables or frequencies for discontinuous variables are reported. Statistical significance of the difference between groups of patients and controls was assessed by Student's *t*-test (for continuous variables) or chi-square test (for categorical variables) and the P values are indicated. Ninety-one patients and 61 controls were included in the study. Among these patients and controls, there were more women than men, except for the CKD4-ND group. The average age of PD patients was 51 ± 16 years, slightly higher than that of CKD (49 ± 18 years) and HD (47 ± 17 years) patients. Because the controls were significantly younger than patients, we divided the control group into two subgroups, with the older group being in the age range of the patients. And as the younger age group (with tail DNA intensity, 11.1 ± 1.1%) behaved in a statistically homogeneous manner as a total control (TC, with tail DNA intensity, 11.2 ± 1.1%), we decided to refer only to the TC group for tail DNA intensity and the older age group for MN frequencies. All patients were classified for CKD stage according to the NKF-KDOQI guidelines (32). The presence of diabetes mellitus (DM) differed among the patient groups and was 52% in the CKD4-ND group, 48% in the PD group and 37% in the HD group. Body mass index was significantly higher in PD patients and the older controls. The differences in the routine biochemical parameters were as expected: creatinine and urea were higher in dialysis groups when compared with CKD4-ND and controls. Haemoglobin and serum albumin levels were lower.

Inflammation and oxidative stress markers

Inflammation markers, such as hs-CRP, IL-6 and TNF-α, were increased in patients when compared with older controls (oCs; hs-CRP: 0.8 ± 0.1; IL-6: 2.0 ± 0.3; TNF-α: 2.7 ± 0.7); the highest levels of inflammation markers were found in HD patients

Table I. Clinical and analytical data for CKD pre-dialysis and dialysis (PD or HD) patients and controls

	TC	yC	oC	CKD4-ND	PD	HD
n	61	48	13	23	33	35
Age (years)	37 ± 9	33.9 ± 5.8^{a1}	49.1 ± 5.7	49 ± 18^{b}	51 ± 16^{b}	47 ± 17^{b}
Gender (male/female)	27/33	27/21	6/7	12/11^{b,c}	13/20	15/20
Diabetes (%)	0	0	0	52.2^{b}	48.5^{b}	37.1^{b}
BMI (kg/m^2)	25.5 ± 2.9	25.3 ± 2.8	36.3 ± 3.5	25.3 ± 3.6	26.2 ± 4.3	23.8 ± 3.8
Urea (mg/dl)	28.5 ± 6.4	27.7 ± 6.4	31.0 ± 5.6	57.4 ± 51^{b,c,e}	86.5 ± 37.4^{b}	83.3 ± 34.8^{b}
Creatinine (mg/dl)	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	2.8 ± 1.4^{b,d,f}	7.0 ± 3.5^{b,b1}	7.2 ± 2.7^{b,b1}
eGFR (ml/min)	114.1 ± 22.5	115 ± 28.2	106 ± 20.2	22.5 ± 14.9	0	0
Total cholesterol (mg/dl)	200 ± 37	196 ± 37	216 ± 36	183 ± 34^{b,d}	188 ± 38^{e}	151 ± 35^{b,b1}
HDL cholesterol (mg/dl)	49 ± 13	49 ± 14	49 ± 11	43 ± 14	45 ± 13	44 ± 128
LDL cholesterol (mg/dl)	118 ± 37	114 ± 36	135 ± 34	103 ± 27^{a1}	99 ± 19^{a,b1}	91 ± 20^{b,b1}
Triglycerides (mg/dl)	142 ± 66	140 ± 67	155 ± 62	157 ± 52	198 ± 142^{a}	121 ± 60^{f}
Serum albumin (g/dl)	4.9 ± 0.3	4.9 ± 0.3	5 ± 0.1	4.0 ± 0.7^{b,b1}	3.7 ± 0.5^{b,b1}	3.8 ± 0.7^{b,b1}
Calcium (mg/dl)	9.2 ± 0.5	9.2 ± 0.5	9.3 ± 0.5	9.1 ± 0.6	9.5 ± 0.7	9.2 ± 0.8
Phosphorus (mg/dl)	4.3 ± 0.7	4.4 ± 0.6	4.2 ± 0.8	3.1 ± 1.1^{b,d,f}	5.1 ± 1.5^{a}	5.4 ± 1.5^{b,a1}
Haemoglobin (g/dl)	15.6 ± 1.6	15.7 ± 1.7	15.2 ± 1.1	11.0 ± 1.9^{b,b1}	10.7 ± 2.5^{b,b1}	10.4 ± 2.3^{b,b1}
Leucocytes (1×10^3/mm^3)	6.7 ± 1.5	6.8 ± 4.5	6.5 ± 1.3	6.9 ± 2.3	6.9 ± 1.3	6.2 ± 1.9
Inflammation markers						
hs-CRP (mg/dl)	0.7 ± 0.2	0.6 ± 0.2	0.8 ± 0.1	3.5 ± 5.0^{a}	4.4 ± 3.6^{b,a1}	5.9 ± 5.3^{b,b1}
IL-6 (pg/ml)	2.0 ± 1.0	2.1 ± 1.1	2.0 ± 0.3	4.3 ± 3.4^{a,e}	4.7 ± 3.0^{a,a1}	6.2 ± 3.6^{a,b1}
TNF-α (pg/ml)	3.1 ± 1.7	3.2 ± 1.8	2.7 ± 0.7	4.4 ± 2.9^{b,e}	5.4 ± 3.1^{b,a1}	6.9 ± 3.9^{b,b1}
Oxidative stress markers						
8-OHdG (ng/ml)	26.9 ± 8.0	27.8 ± 7.5	23.4 ± 9.2	43.2 ± 11.3^{b,f,b1}	29.9 ± 11.5^{d}	23.9 ± 13.6^{d}
AGEs (AU/mg protein)	2.0 ± 0.5	2.0 ± 0.4	2.1 ± 0.7	4.7 ± 2.1^{b,e,b1}	5.0 ± 2.3^{b,c,b1}	6.5 ± 2.4^{b,b1}
Thiols (mmol/l)	35.8 ± 19	35.4 ± 19	39.8 ± 19	47.2 ± 31.7	58.0 ± 44.0^{b}	52.2 ± 35.0
AOPP (µmol/l)	33.2 ± 11	33.2 ± 11	34.4 ± 11	37.7 ± 10.8^{b}	43.6 ± 8.0^{a}	37.3 ± 14.8
Malondialdehyde (µmol/l)	4.6 ± 0.9	4.5 ± 0.8	4.7 ± 0.9	6.6 ± 1.7^{b,e,a1}	6.0 ± 2.0^{b}	5.3 ± 1.7
DNA and chromosome damage markers						
Comet assay tail DNA intensity (%)	11.2 ± 1.1	11.1 ± 1.1	11.6 ± 1.4	15.6 ± 7.1^{b}	14.8 ± 7.4^{b}	12.5 ± 3.2
Micronuclei/1000 BNC (‰)	24.4 ± 9.5	24.4 ± 10	27.7 ± 14	46.2 ± 4.3^{a,f,a1}	41.9 ± 14.0^{a,f,a1}	29.7 ± 15.6

TC, total control; yC, young controls; oC, older controls; CKD4-ND, CKD stage 4 non-dialysed; PD, peritoneal dialysis; HD, haemodialysis; BMI, body mass index; eGFR, estimated glomerular filtration from creatinine clearance (Cockoft–Gault formula); HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AGE, advanced glycation end products; AOPP, advanced oxidation protein products (AOPP concentrations are expressed as µmol/l of chloramine-T); BNC, binucleated cells; AU, arbitrary units; P values are based on Student's *t*-test or chi-square according to the variable characteristics; $^{a}P < 0.05$ versus TC; $^{b}P < 0.001$ versus TC; $^{a1}P < 0.05$ versus oC; $^{b1}P < 0.001$ versus oC; $^{c}P < 0.05$ versus PD; $^{d}P < 0.001$ versus PD; $^{e}P < 0.05$ versus HD; $^{f}P < 0.001$ versus HD. Values are mean ± standard deviation (SD).

(hs-CRP: 5.9±5.3, $P < 0.001$; IL-6: 6.2±3.6, $P < 0.001$; TNF-α: 6.9±3.9, $P < 0.001$) (Table I). Oxidative stress, measured by the concentration of AGEs, AOPP, thiols and MDA, was also generally higher in dialysis patients (AGEs: 5.0±2.3, $P < 0.05$; thiols: 58±44, P = NS; AOPP: 43.6±8.0, P = NS; MDA: 6±2, P = NS) when compared with CKD4-ND (AGEs: 4.7±2.1, $P < 0.05$; thiols: 47.2±31.7, P = NS; AOPP: 37.7±10.8, P = NS; MDA: 6.6±1.7, P = NS), and both dialysed and non-dialysed patients had higher values than both controls (TC: AGEs = 2±0.5; thiols: 35.8±19; AOPP: 33.2±11; MDA: 4.6±0.9 and oC: AGEs = 2.1±0.7; thiols: 39.8±19; AOPP: 34.4±11; MDA: 4.7±0.9). 8-OHdG levels were higher in CKD4-ND patients (43.2±11.3, $P < 0.001$) compared with TCs (26.9±8.0) (Table I). However, AOPPs were higher in both CKD4-ND (37.7±10.8, $P < 0.001$) and PD (43.6±8.0, $P < 0.05$), but not HD patients (37.3±14.8, P = NS), when compared with TCs (33.2±11). Significant correlations were observed between variables of oxidative stress and inflammation markers (Table II).

DNA and chromosome damage markers

Table I also shows all MN frequencies scored (for CKD patients = 46±4‰, $P < 0.05$; PD = 41.9±14‰, $P < 0.05$; HD = 29.7±16‰, P = NS, in comparison with the oC = 27.7±14‰), and the results obtained in the comet assay, which showed a significant increase ($P < 0.001$) in tail DNA intensity in cells of patients with CKD (15.6±7%) compared with the TC group (11.1±7%) followed by PD (14.8±7%), which also have a significant increase ($P < 0.001$) versus TC and HD (12.5±3%, P = NS) as compared with the TC group. Table II summarises significant correlations between the investigated markers; of these, MDA and 8-OHdG had stronger correlations with MN frequency.

To analyse the impact of the level of chromosome damage based on MN frequency in PBL, patients were divided into two groups: with high MN frequencies (n = 45) and with low MN frequencies (n = 46), taking as cut-off point the value of 35 MN/1000 BNC (upper quartile) of CBMN assay (Table III). Total cholesterol, triglycerides, AOPP and MDA were significantly higher in patients with high MN frequencies levels. Patients with low levels of MN frequencies in general had lower levels of IL-6, TNF-α and AGEs.

In order to analyse which factors were independent predictors of the background level of DNA effects in PBL, a logistic correlation test was performed (Table IV). In all models, the dependent variable was the MN frequency. The patients were classified into six groups according to CKD stage, treatment modality and the presence or absence of DM: Group 1 = CKD4-ND/DM−, Group 2 = CKD4-ND/DM+, Group 3 = PD/DM−, Group 4 = PD/DM+, Group 5 = HD/DM− and Group 6 = HD/DM+ (reference group). When DM within groups of treatment was introduced as independent variable in the regression model, we observed significance in Groups 2–4 (reference group HD/DM+ with DM). Variables that were independently related to background levels of DNA effects in PBL were DM within groups of treatment, male gender and a high hs-CRP value.

Discussion

The current study confirms that patients with advanced CKD have increased DNA and chromosome damage in PBL measured with the CBMN assay and the comet assay, which putatively could be assumed to be linked to the high incidence of cardiovascular disease and cancer among these patients (2,33).

Although non-genetic factors such as suppression of the immune system, chronic inflammation, viral-associated factors, increased levels of oxidative stress, reduced antioxidant levels and accumulation of uraemic toxins are thought to contribute to the high morbidity in patients with ESRD, this could conceivably be, at least in part, mediated by genetic damage. Various biological parameters have been measured to determine the burden of DNA damage, like single- and double-strand breaks and alkali-labile sites, detectable with the comet assay, which have been found to be significantly increased in PBL of patients undergoing dialysis (17,22). These types of DNA damage may be repaired. Likewise, previous studies—using

Table II. Correlations between the different parameters of oxidative stress expressed as Pearson correlation coefficients and corresponding P values

	8-OHdG	AGES	AOPP	MDA	Thiols	hs-CRP	IL-6	TNF-α	MN	CATI
8-OHdG	1.000	−0.038	−0.001	**0.366****	−0.011	−0.035	−0.048	−0.094	**0.227****	0.142
		0.642	0.987	0.000	0.897	0.670	0.562	0.251	0.005	0.083
AGEs	−0.038	1.000	0.051	**0.194***	**0.274****	**0.353****	**0.435****	**0.394****	0.026	0.093
	0.642		0.531	0.017	0.001	0.000	0.000	0.000	0.747	0.256
AOPP	−0.001	0.051	1.000	**0.231****	0.158	0.005	0.024	0.016	**0.161***	0.134
	0.987	0.531		0.004	0.052	0.948	0.771	0.814	0.047	0.100
MDA	***0.366****	***0.194****	***0.231****	1.000	−0.079	0.024	0.018	0.022	***0.318****	0.140
	0.000	0.017	0.004		0.336	0.766	0.825	0.786	0.000	0.085
Thiols	−0.011	***0.274****	0.158	−0.079	1.000	***0.258****	***0.317****	***0.272****	0.003	***0.178****
	0.897	0.001	0.052	0.336		0.001	0.000	0.001	0.974	0.029
hs-CRP	−0.035	***0.353****	0.005	0.024	***0.258****	1.000	***0.682****	***0.639****	0.117	0.059
	0.670	0.000	0.948	0.776	0.001		0.000	0.000	0.152	0.472
IL-6	−0.048	***0.435****	0.024	0.018	***0.317****	***0.682****	1.000	***0.915****	0.059	0.102
	0.562	0.000	0.771	0.825	0.000	0.000		0.000	0.467	0.210
TNF-α	−0.094	***0.394****	0.016	0.022	***0.272****	***0.639****	***0.915****	1.000	0.033	0.053
	0.251	0.000	0.843	0.786	0.001	0.000	0.000		0.688	0.512
MN	***0.227****	0.026	***0.161****	***0.318****	0.003	0.117	0.059	0.033	1.000	***0.250****
	0.005	0.747	0.047	0.000	0.974	0.152	0.467	0.688		0.002
CATI	0.142	0.093	0.134	0.140	***0.178****	0.059	0.102	0.053	***0.250****	1.000
	0.083	0.256	0.100	0.085	0.029	0.472	0.210	0.513	0.002	

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AGES, advanced glycation end product; AOPP, advanced oxidation protein products; MDA, malondialdehyde; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha; MN, micronucleus; CATI, comet assay tail DNA intensity. Numbers in bold and italics indicate significant correlations. **Correlation is significant at the 0.01 level (two-tailed). *Correlation is significant at the 0.05 level (two-tailed).

Table III. Clinical and analytical characteristics of patients defined by upper quartile of the CBMN assay

	Low MN frequencies (n = 46), MN < 35 ‰	High MN frequencies (n = 45), MN ≥ 35‰	P
N	46	45	
Age (years)	48 ± 16	50 ± 17	NS
Diabetes (%)	43.5	46.7	NS
BMI (kg/m^2)	24.8 ± 3.6	25.3 ± 4.5	NS
Urea (mg/dl)	74.9 ± 44.2	80.7 ± 39.0	NS
Creatinine (mg/dl)	6.3 ± 3.4	5.6 ± 3.3	NS
Total cholesterol (mg/dl)	164 ± 34	181 ± 43	0.030
HDL cholesterol (mg/dl)	44 ± 12	44.9 ± 14.3	NS
LDL cholesterol (mg/dl)	94.7 ± 21.5	99 ± 23	NS
Triglycerides (mg/dl)	137 ± 67	178 ± 124	0.050
Serum albumin (g/dl)	3.9 ± 0.7	3.8 ± 0.6	NS
Serum Ca (mg/dl)	9.3 ± 0.7	9.3 ± 0.8	NS
Phosphorus (mg/dl)	4.7 ± 1.9	4.7 ± 1.4	NS
Haemoglobin (g/dl)	10.5 ± 2.2	10.9 ± 2.3	NS
hs-CRP (mg/dl)	5.6 ± 5.5	3.9 ± 3.7	NS
IL-6 (pg/ml)	6.1 ± 3.6	4.2 ± 2.9	0.010
TNF-α (pg/ml)	6.5 ± 3.8	4.9 ± 3.0	0.030
8-OHdG (ng/ml)	28.5 ± 15.0	33.5 ± 13.3	NS
AGEs (AU/mg protein)	6.3 ± 2.4	4.7 ± 2.1	0.001
Thiols (mmol/l)	56.5 ± 37.3	49.5 ± 38.0	NS
AOPP (µmol/l¥)	38.0 ± 15.6	41.5 ± 15.0	NS
Malondialdehyde (µmol/l)	5.5 ± 2.2	6.3 ± 1.4	0.030
Comet assay tail DNA intensity (%)	12.2 ± 3.5	16.0 ± 7.6	0.010
Micronuclei/1000 BNC (‰)	23.7 ± 6.4	53.2 ± 15.3	0.001

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AGE, advanced glycation end product; AU, arbitrary units; AOPP, advanced oxidation protein products; BNC, binucleated cells.

different techniques for the measurement of DNA and chromosome damage (such as MN test, comet assay, measuring of 8-OHdG quantified by high-performance liquid chromatography by electrochemical detection and ELISA, finally mitochondrial DNA deletions by PCR)—have shown that high levels of genetic damage are more pronounced in CKD patients than in populations with normal renal function (5,17,18,22,34–39).

Our results confirm that whereas the presence of DNA and chromosome damage level, measured by comet assay and CBMN assay, is increased in CKD patients, both with and without dialysis treatment, the non-dialysed CKD patients had higher levels of genetic damage than patients on dialysis, suggesting that some molecules removed by dialysis may have a role in the increased genetic damage level. Furthermore, higher levels of genetic damage were associated with inflammation and diabetes. We found higher MN frequencies in the non-dialysed CKD4-ND patients compared with subjects on dialysis and healthy controls. This agrees with Stopper *et al.* (5), who found that in comparison with healthy controls, MN in PBL is increased approximately 2-fold in pre-dialysis CKD patients. Our study concurs with results in previous studies showing increased cytogenetic effects with regards to MN frequencies in pre-dialysis CKD and in patients with a different modality of dialysis treatment (5,14,39–41).

MN mainly originates from acentric chromosome fragments, acentric chromatid fragments or whole chromosomes that fail to be included in the daughter nuclei at the completion of telophase during mitosis because they did not attach properly with the spindle during the segregation process in anaphase. Increased frequency of MNs has been considered to be a good indicator of genetic instability, and thus MNs have been proposed as a surrogate biomarker of cancer risk (6).

The stage of CKD, dialysis modality and co-morbidities seem to influence the background levels of DNA effects in PBL in several ways. The degree of chromosome damage is associated with the degree of impairment of renal function (17), and even

Table IV. Logistic correlation test with the variables related to chromosomal damage (assessed by MN frequency)

Variable	B	SE	Wald	df	Significance	R	Variable	95% CI for Exp(B)		
								Exp(B)	Lower	Upper
Reference group			13.85	5	0.017	0.199				
Group 1	0.85	1.59	0.29	1	0.592	0.000	Group 1	2.35	0.10	53.34
Group 2	3.03	1.35	5.00	1	0.025	0.175	Group 2	20.67	1.45	294.09
Group 3	2.80	1.24	5.08	1	0.024	0.178	Group 3	16.38	1.44	186.18
Group 4	4.64	1.38	11.24	1	0.001	0.307	Group 4	103.72	6.88	1564.06
Group 5	1.87	1.19	2.50	1	0.114	0.071	Group 5	6.51	0.64	66.43
Males	−1.37	0.65	4.37	1	0.037	−0.156	Males	0.26	0.07	0.92
hs-CRP (>1 mg/dl)	−2.56	1.08	5.64	1	0.018	−0.193	hs-CRP (>1 mg/dl)	0.08	0.01	0.64
Constant	−2.94	2.71	1.18	1	0.277					

Reference group: treatment/DM+; Group 1, CKD4-ND/DM−; Group 2, CKD4-ND/DM+; Group 3; PD/DM−; Group 4; PD/DM+; Group 5, HD/DM−. + denotes the presence of DM, − denotes the absence of DM.
HD, haemodialysis; DM, diabetes mellitus; hs-CRP, high sensitivity C-reactive protein; CKD4, chronic kidney disease stage 4 according to the NKF-KDOQI guidelines; ND, non-dialysed.

in patients receiving dialysis; the residual renal function affects the magnitude of this damage. In agreement with previous studies (42–46), our data show more extensive DNA damage in the non-dialysed CKD patients. This suggests that removal of some uraemic toxins by dialysis could reduce the background levels of DNA damage in PBL; however, we did not measure any specific uraemic toxins and we are unable to confirm links between markers of uraemia and level of genetic damage. Regarding the effect of different dialysis treatment modalities, some studies report higher level chromosome damage in PD over HD (17,28,29), while the opposite is found in other studies (47–49). Daily HD and HD with experimental membranes were found to be associated with a lesser degree of DNA damage (50,51). However, it is likely that DNA damage is mainly caused by other factors present in CKD such as oxidative stress (14,52) and not directly related to dialysis modality or type of dialyser membrane.

Dialysis modality influences oxidative stress marker patterns and magnitude. For example, plasma thiobarbituric acid reactive substances, oxidised LDL, asymmetric dimethylarginine and homocysteine levels have been reported to be higher in HD patients than in PD patients and both modalities have higher levels of these compounds than healthy controls (49,53–55). There are, however, some studies where the difference was in the opposite direction (PD > HD) when markers like homocysteine, plasma hydroperoxides, total antioxidant defence and erythrocyte plasma membrane fluidity were measured (56,57).

Not all oxidative stress markers have the same meaning. AGEs are more closely related with metabolic control and diabetes and are well established as species triggering inflammation. Diabetes, residual renal function and dialysis dose were not uniformly distributed within the investigated groups. Diabetes and higher age, two factors with known effect on the level of genetic damage, were more frequent in PD than in HD patients. This may explain why, in this study, HD patients had lower levels of genetic damage than PD patients.

The presence of a low-degree chronic inflammatory state has been widely documented in CKD patients. In the present study, significant correlations were found among markers of oxidative stress, as well as between inflammation markers, and between oxidative stress markers and inflammation markers, emphasizing the link between both processes (58,59). 8-OHdG was significantly correlated with MDA, as well as with MN. 8-OHdG is a marker of intracellular oxidative stress (35,36,38).

The presence of diabetes in CKD patients is associated with inflammation and oxidative stress when compared with non-diabetic patients, and we found significant differences in this regard. However, possibly due to the heterogeneity of the patients, the effect of diabetes was only seen when the analysis was controlled by treatment modality.

Limitations of our study include its cross-sectional design, which precludes conclusions regarding possible cause–effect relationships, the small number of patients and lack of detailed clinical characteristics of the investigated cohorts. Nevertheless, the sample size was sufficient to find differences, which were statistically related with diabetic status, modality of CKD treatment and the presence of low-degree chronic inflammation. The potential negative effects of the degree of genetic damage, e.g. on the development of malignancies in renal patients, were not investigated in this cohort. Studies with larger populations and longer follow-up periods are needed to address whether the level of genetic damage has an impact on clinical outcome.

In summary, these results indicate that CKD patients show increased background levels of DNA damage, which indicate reduced DNA integrity in patients with CKD. These changes were more marked in male, diabetic and non-dialysed stage 4 CKD patients, while CKD stage 5 patients undergoing HD treatment appeared to have fewer alterations compared with those treated by PD. To confirm these data, prospective clinical trials need to be performed.

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References

1. Weiner, D. E. (2007) Causes and consequences of chronic kidney disease: implications for managed health care. *J. Manag. Care Pharm.*, **13**, S1–S9.
2. Maisonneuve, P., Agodoa, L., Gellert, R., *et al.* (1999) Cancer in patients on dialysis for end-stage renal disease: an international collaborative study. *Lancet*, **354**, 93–99.
3. Sebekova, K., Wagner, Z., Schupp, N. and Boor, P. (2007) Genomic damage and malignancy in end-stage renal failure: do advanced glycation end products contribute? *Kidney Blood Press. Res.*, **30**, 56–66.
4. Schupp, N., Stopper, H., Rutkowski, P., Kobras, K., Nebel, M., Bahner, U., Vienken, J. and Heidland, A. (2006) Effect of different hemodialysis regimens on genomic damage in end-stage renal failure. *Semin. Nephrol.*, **26**, 28–32.
5. Stopper, H., Meysen, T., Bockenforde, A., Bahner, U., Heidland, A. and Vamvakas, S. (1999) Increased genomic damage in lymphocytes of patients before and after long-term maintenance hemodialysis therapy. *Am. J. Kidney Dis.*, **34**, 433–437.
6. Bonassi, S., El-Zein, R., Bolognesi, C. and Fenech, M. (2011) Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. *Mutagenesis*, **26**, 93–100.
7. Himmelfarb, J. (2009) Uremic toxicity, oxidative stress, and hemodialysis as renal replacement therapy. *Semin. Dial.*, **22**, 636–643.
8. Schupp, N., Heidland, A. and Stopper, H. (2010) Genomic damage in end-stage renal disease—contribution of uremic toxins. *Toxins*, **2**, 2340–2358.
9. Vaziri, N. D. (2004) Oxidative stress in uremia: nature, mechanisms, and potential consequences. *Semin. Nephrol.*, **24**, 469–473.
10. Hussain, S. P. and Harris, C. C. (2007) Inflammation and cancer: an ancient link with novel potentials. *Int. J. Cancer*, **121**, 2373–2380.
11. Colotta, F., Allavena, P., Sica, A., Garlanda, C. and Mantovani, A. (2009) Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*, **30**, 1073–1081.
12. Noordzij, M. J., Lefrandt, J. D. and Smit, A. J. (2008) Advanced glycation end products in renal failure: an overview. *J. Ren. Care*, **34**, 207–212.
13. Meerwaldt, R., Zeebregts, C. J., Navis, G., Hillebrands, J. L., Lefrandt, J. D. and Smit, A. J. (2009) Accumulation of advanced glycation end products and chronic complications in ESRD treated by dialysis. *Am. J. Kidney Dis.*, **53**, 138–150.
14. Kobras, K., Schupp, N., Nehrlich, K., Adelhardt, M., Bahner, U., Vienken, J., Heidland, A., Sebekova, K. and Stopper, H. (2006) Relation between different treatment modalities and genomic damage of end-stage renal failure patients. *Kidney Blood Press. Res.*, **29**, 10–17.
15. Speit, G., Linsenmeyer, R., Schütz, P. and Kuehner, S. (2012) Insensitivity of the in vitro cytokinesis-block micronucleus assay with human lymphocytes for the detection of DNA damage present at the start of the cell culture. *Mutagenesis*, **27**, 743–747.
16. Fenech, M. (1993) The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations. *Environ. Health Perspect.*, **101** (Suppl. 3), 101–107.

17. Stopper, H., Boullay, F., Heidland, A., Vienken, J. and Bahner, U. (2001) Comet-assay analysis identifies genomic damage in lymphocytes of uremic patients. *Am. J. Kidney Dis.*, **38**, 296–301.
18. Tarng, D. C., Huang, T. P., Wei, Y. H., Liu, T. Y., Chen, H. W., Wen Chen, T. and Yang, W. C. (2000) 8-Hydroxy-2′-deoxyguanosine of leukocyte DNA as a marker of oxidative stress in chronic hemodialysis patients. *Am. J. Kidney Dis.*, **36**, 934–944.
19. Hsu, G. W., Ober, M., Carell, T. and Beese, L. S. (2004) Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase. *Nature*, **431**, 217–221.
20. Rodier, F., Campisi, J. and Bhaumik, D. (2007) Two faces of p53: aging and tumor suppression. *Nucleic Acids Res.*, **35**, 7475–7484.
21. Jackson, A. L. and Loeb, L. A. (1998) The mutation rate and cancer. *Genetics*, **148**, 1483–1490.
22. Kan, E., Undeger, U., Bali, M. and Basaran, N. (2002) Assessment of DNA strand breakage by the alkaline COMET assay in dialysis patients and the role of vitamin E supplementation. *Mutat. Res.*, **520**, 151–159.
23. Vamvakas, S., Bahner, U., Becker, P., Steinle, A., Götz, R. and Heidland, A. (1996) Impairment of DNA repair in the course of long-term hemodialysis and under cyclosporine immunosuppression after renal transplantation. *Transplant. Proc.*, **28**, 3468–3473.
24. Herbert, K. E., Mistry, Y., Hastings, R., Poolman, T., Niklason, L. and Williams, B. (2008) Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. *Circ. Res.*, **102**, 201–208.
25. Li, H., Mitchell, J. R. and Hasty, P. (2008) DNA double-strand breaks: a potential causative factor for mammalian aging? *Mech. Ageing Dev.*, **129**, 416–424.
26. Mercer, J., Mahmoudi, M. and Bennett, M. (2007) DNA damage, p53, apoptosis and vascular disease. *Mutat. Res.*, **621**, 75–86.
27. Loft, S. and Poulsen, H. E. (1996) Cancer risk and oxidative DNA damage in man. *J. Mol. Med. (Berl.)*, **74**, 297–312.
28. Buemi, M., Floccari, F., Costa, C., *et al.* (2006) Dialysis-related genotoxicity: sister chromatid exchanges and DNA lesions in T and B lymphocytes of uremic patients. Genomic damage in patients on hemodiafiltration. *Blood Purif.*, **24**, 569–574.
29. Roth, J. M., Restani, R. G., Goncalves, T. T., Sphor, S. L., Ness, A. B., Martino-Roth, M. G. and Garcias, G. L. (2008) Genotoxicity evaluation in chronic renal patients undergoing hemodialysis and peritoneal dialysis, using the micronucleus test. *Genet. Mol. Res.*, **7**, 433–443.
30. Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider, E. L. (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.*, **175**, 184–191.
31. Fenech, M., Chang, W. P., Kirsch-Volders, M., Holland, N., Bonassi, S. and Zeiger, E.; HUman MicronNucleus project. (2003) HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat. Res.*, **534**, 65–75.
32. Foundation NK (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am. J. Kidney Dis.*, **39**, S1–S266.
33. Herzog, C. A., Ma, J. Z. and Collins, A. J. (1998) Poor long-term survival after acute myocardial infarction among patients on long-term dialysis. *N. Engl. J. Med.*, **339**, 799–805.
34. Liu, C. S., Ko, L. Y., Lim, P. S., Kao, S. H. and Wei, Y. H. (2001) Biomarkers of DNA damage in patients with end-stage renal disease: mitochondrial DNA mutation in hair follicles. *Nephrol. Dial. Transplant.*, **16**, 561–565.
35. Akagi, S., Nagake, Y., Kasahara, J., *et al.* (2003) Significance of 8-hydroxy-2′-deoxyguanosine levels in patients with chronic renal failure. *Nephrology (Carlton)*, **8**, 192–195.
36. Domenici, F. A., Vannucchi, M. T., Jordao, A. A., Jr, Meirelles, M. S. and Vannucchi, H. (2005) DNA oxidative damage in patients with dialysis treatment. *Ren. Fail.*, **27**, 689–694.
37. Bagatini, P. B., Palazzo, R. P., Rodrigues, M. T., Costa, C. H. and Maluf, S. W. (2008) Induction and removal of DNA damage in blood leukocytes of patients with type 2 diabetes mellitus undergoing hemodialysis. *Mutat. Res.*, **657**, 111–115.
38. Puchades Montesa, M. J., Gonzalez Rico, M. A., Solis Salguero, M. A., Torregrosa Maicas, I., Tormos Munoz, M. C., Saez Tormo, G., Juan Garcia, I. and Miguel Carrasco, A. (2009) [Study of oxidative stress in advanced kidney disease]. *Nefrologia*, **29**, 464–473.
39. Sandoval, S. B., Stoyanova, E., Coll, E., Pastor, S., Reyes, J., Andres, E., Ballarin, J., Xamena, N. and Marcos, R. (2010) Genetic damage in chronic renal failure patients is associated with the glomerular filtration rate index. *Mutagenesis*, **25**, 603–608.
40. Stopper, H., Treutlein, A. T., Bahner, U., Schupp, N., Schmid, U., Brink, A., Perna, A. and Heidland, A. (2008) Reduction of the genomic damage level in haemodialysis patients by folic acid and vitamin B12 supplementation. *Nephrol. Dial. Transplant.*, **23**, 3272–3279.
41. Fragedaki, E., Nebel, M., Schupp, N., *et al.* (2005) Genomic damage and circulating AGE levels in patients undergoing daily versus standard haemodialysis. *Nephrol. Dial. Transplant.*, **20**, 1936–1943.
42. Filiopoulos, V., Hadjiyannakos, D., Metaxaki, P., Sideris, V., Takouli, L., Anogiati, A. and Vlassopoulos, D. (2008) Inflammation and oxidative stress in patients on hemodiafiltration. *Am. J. Nephrol.*, **28**, 949–957.
43. Malachi, T., Zevin, D., Gafter, U., Chagnac, A., Slor, H. and Levi, J. (1993) DNA repair and recovery of RNA synthesis in uremic patients. *Kidney Int.*, **44**, 385–389.
44. Dounousi, E., Papavasiliou, E., Makedou, A., Ioannou, K., Katopodis, K. P., Tselepis, A., Siamopoulos, K. C. and Tsakiris, D. (2006) Oxidative stress is progressively enhanced with advancing stages of CKD. *Am. J. Kidney Dis.*, **48**, 752–760.
45. Terawaki, H., Yoshimura, K., Hasegawa, T., *et al.* (2004) Oxidative stress is enhanced in correlation with renal dysfunction: examination with the redox state of albumin. *Kidney Int.*, **66**, 1988–1993.
46. Locatelli, F., Canaud, B., Eckardt, K. U., Stenvinkel, P., Wanner, C. and Zoccali, C. (2003) Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. *Nephrol. Dial. Transplant.*, **18**, 1272–1280.
47. Fenton, S. S., Schaubel, D. E., Desmeules, M., Morrison, H. I., Mao, Y., Copleston, P., Jeffery, J. R. and Kjellstrand, C. M. (1997) Hemodialysis versus peritoneal dialysis: a comparison of adjusted mortality rates. *Am. J. Kidney Dis.*, **30**, 334–342.
48. Oreopoulos, D. G., Ossareh, S. and Thodis, E. (2008) Peritoneal dialysis: past, present, and future. *Iran. J. Kidney Dis.*, **2**, 171–182.
49. Mekki, K., Taleb, W., Bouzidi, N., Kaddous, A. and Bouchenak, M. (2010) Effect of hemodialysis and peritoneal dialysis on redox status in chronic renal failure patients: a comparative study. *Lipids Health Dis.*, **9**, 93.
50. Muller, C., Eisenbrand, G., Gradinger, M., *et al.* (2004) Effects of hemodialysis, dialyser type and iron infusion on oxidative stress in uremic patients. *Free Radic. Res.*, **38**, 1093–1100.
51. Tarng, D. C., Wen Chen, T., Huang, T. P., Chen, C. L., Liu, T. Y. and Wei, Y. H. (2002) Increased oxidative damage to peripheral blood leukocyte DNA in chronic peritoneal dialysis patients. *J. Am. Soc. Nephrol.*, **13**, 1321–1330.
52. Tarng, D. C., Liu, T. Y. and Huang, T. P. (2004) Protective effect of vitamin C on 8-hydroxy-2′-deoxyguanosine level in peripheral blood lymphocytes of chronic hemodialysis patients. *Kidney Int.*, **66**, 820–831.
53. Capusa, C., Stoian, I., Rus, E., Lixandru, D., Barbulescu, C. and Mircescu, G. (2012) Does dialysis modality influence the oxidative stress of uremic patients? *Kidney Blood Press. Res.*, **35**, 220–225.
54. Samouilidou, E. C., Karpouza, A. P., Kostopoulos, V., Bakirtzi, T., Pantelias, K., Petras, D., Tzanatou-Exarchou, H. and J Grapsa, E. (2012) Lipid abnormalities and oxidized LDL in chronic kidney disease patients on hemodialysis and peritoneal dialysis. *Ren. Fail.*, **34**, 160–164.
55. Eiselt, J., Rajdl, D., Racek, J., Siroká, R., Trefil, L. and Opatrná, S. (2010) Asymmetric dimethylarginine in hemodialysis, hemodiafiltration, and peritoneal dialysis. *Artif. Organs*, **34**, 420–425.
56. Filiopoulos, V., Hadjiyannakos, D., Takouli, L., Metaxaki, P., Sideris, V. and Vlassopoulos, D. (2009) Inflammation and oxidative stress in end-stage renal disease patients treated with hemodialysis or peritoneal dialysis. *Int. J. Artif. Organs*, **32**, 872–882.
57. Goi, G., Massaccesi, L., Baquero Herrera, C. J., Musetti, C., Ciurlino, D., Cusi, D. and Bertoli, S. (2009) Oxidative stress in elderly chronic renal failure patients: effects of renal replacement therapies on cell membrane fluidity. *J. Nephrol.*, **22**, 630–636.
58. Oberg, B. P., McMenamin, E., Lucas, F. L., McMonagle, E., Morrow, J., Ikizler, T. A. and Himmelfarb, J. (2004) Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int.*, **65**, 1009–1016.
59. Kao, M. P., Ang, D. S., Pall, A. and Struthers, A. D. (2010) Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. *J. Hum. Hypertens.*, **24**, 1–8.