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Levels of DNA damage in peripheral blood lymphocytes of patients undergoing standard hemodialysis *vs* on-line hemodiafiltration: A comet assay investigation

Zuray Corredor[a], Lara Rodríguez-Ribera[a], Irene Silva[b], Juan Manuel Díaz[b], José Ballarín[b], Ricard Marcos[a,c], Elisabet Coll[b,1], Susana Pastor[a,c,*,1]

[a] *Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain*
[b] *Fundació Puigvert, Barcelona, Spain*
[c] *CIBER Epidemiología y Salud Pública, ISCIII, Madrid, Spain*

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ABSTRACT

Chronic kidney disease (CKD) patients exhibit high levels of genetic damage. Part of this genetic damage is supposed to be caused by the hemodialysis (HD) therapy. Different and more efficient HD procedures could reduce the genetic damage and improve health status of CKD patients. In the present study, we analyzed if changing to online hemodiafiltration (OL-HDF) has a beneficial effect on the levels of genetic damage. The levels of genetic damage (DNA breaks and oxidatively damaged DNA) were analyzed in peripheral blood lymphocytes by using the comet assay. Forty-nine patients submitted to HD, 34 of them changing to OL-HDF and 15 patients continuing in low-flux HD, were included in the study. Plasma antioxidant capacity was also determined. Second sampling period was established after 6 months on the new or traditional HD protocol. A slight decrease in the levels of DNA damage was observed in patients who switched to OL-HDF ($P = 0.048$) in relation to the reference group. This reduction is indicative that OL-HDF shows greater efficiency than low-flux HD in the reduction of basal levels of genetic damage.

1. Introduction

Millions of end-stage renal patients over the world survive thanks to hemodialysis therapies [1]. In chronic kidney disease (CKD) patients with renal failure, conventional hemodialysis (HD) is the main renal replacement modality used to remove solutes [2,3]. Despite the different HD alternatives that exist, patients still suffer several and serious health problems such as malnutrition, diabetes mellitus and cardiovascular diseases, among other, and exhibit high rates of mortality [4], some of these alterations are due to the accumulation of solutes that cannot be removed by the HD techniques.

One of the proposed extracorporeal HD alternatives is the on-line hemodiafiltration (OL-HDF), which combines the advantages of classical hemodialysis (small solute removal, by diffusion) with those of hemofiltration (large solute removal, by convection) [5]. OL-HDF uses high-flux synthetic membranes and ultrapure dialysis fluids that allow the clearance of medium to large molecules that are impossible to remove using exclusively diffusive techniques. On the contrary, low-flux hemodialysis uses membranes with low permeability and exclusively diffusive techniques. OL-HDF showed other advantages such as an improvement of the hemodynamics stability, better removal of phosphates, decrease of amyloidosis and markers of chronic inflammation, and improvements in survival and nutritional status [6]. In fact, four recent studies have found a reduction in mortality with OL-HDF that correlates with the convection volumes achieved during therapy [7–10]. High levels of genomic damage are present in CKD patients and recently this value has been proposed as biomarker of chronic renal disease status [11]. Such high levels of DNA damage can be due to the presence of toxins not removed from blood, such as advanced glycation

Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; FPG, formamidopyrimidine DNA-glycosylase; HD, hemodialysis; OL-HDF, online hemodiafiltration; TIBC, total iron binding capacity; TEAC, the trolox equivalent antioxidant capacity.

* Corresponding author at: Grup de Mutagènesi, Departament de Genètica i de Microbiologia, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Cerdanyola del Vallès, Spain.
E-mail address: susana.pastor@uab.es (S. Pastor).
[1] Co-direct the study.

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end-products (AGEs) [12], or to deficiencies in DNA repair ability [13]. In addition, the high levels of DNA damage present in CKD patients have been directly associated with all-type mortality [14]. Although an important part of the increased level of genomic damage can be associated to the renal pathology itself [2], differences in the HD protocol can also influence the final levels of DNA damage [15]. In this context it seems interesting to evaluate whether moving from conventional low-flux HD to OL-HDF acts as a factor modulating the levels of genomic damage.

With regard to the relevance of individual genomic damage it should be pointed out that there are clear evidences linking genetic damage levels with adverse health outcomes [16]. The accumulation of genomic changes by both endogenous and exogenous factors is recognized as a fundamental underlying cause of developmental defects and accelerated aging as well as of an increased risk of degenerative conditions such as infertility, immune dysfunction, cancer, and cardiovascular and neurodegenerative disease [17].

In a previous study we observed that patients with chronic kidney disease (CKD), which were changed to OL-HDF therapy, showed a significant reduction of the basal levels of genetic damage using the micronucleus assay, that detects both structural chromosomal breakage as well as aneuploidy [18]. In addition, a greater increase in plasma antioxidant capacity was detected with the Trolox equivalent antioxidant capacity (TEAC) assay [18]. In the present study we extend the previous work using the comet assay, in peripheral blood lymphocytes from patients, as a tool to determine the levels of genomic damage. This approach permits detection of DNA strand breaks as well as oxidized DNA bases, when the comet assay is complemented with the use of formamidopyrimidine DNA-glycosylase (FPG) [19].

2. Materials and methods

2.1. Study population

The study was carried out in 49 patients with chronic kidney disease underwent thrice weekly low-flux HD treatment. The inclusion criteria were as follows: aged over 18 years, undergoing HD for more than 3 months who were stable on 3 weekly sessions of 3.5–4 h and a stable regimen of anticoagulation and erythropoietin with a vascular access allowing flows greater than 250 mL/min. Patients with a survival rate lower than 18 months or with an expected kidney transplantation in the next 6 months or with significant residual renal function (diuresis greater than 400 mL/day or creatinine clearance >2 mL/min) were excluded from the study. At the beginning of the study, all patients underwent low flux hemodialysis for 3.5–4 h thrice weekly using conventional bicarbonate concentrate. Moreover, all patients were using low permeable synthetic membranes (Vitapes®BF 200 was a polyetersulphone with an area of 2 m^2 and ultrafiltration ratio of 24 mL/h/mmHg). From the overall group, 34 patients with a previous average period of 26.06 ± 5.28 months submitted to low-flux HD therapy, changed to OL-HDF. Another group of 15 patients, with a previous average period of 17.87 ± 6.98 months submitted to low-flux HD therapy, continued in low-flux HD, and it was used as a reference group. Patients were randomized by a computer program, and those who started OL-HDF had not previously been submitted to OL-HDF. Two blood samples were obtained for each patient: the first one was obtained before the first OL-HDF session and the second one after 6 months on OL-HDF therapy. For the group that remains in conventional HD, the period of time between both samples was of 7.9 months. Blood samples were obtained before the middle dialysis session. When patients changed to on-line HDF, they changed the membranes to Vitapes®HF190 that was a polyetersulphone with an area of 1.9 m^2 and ultrafiltration rate of 80 mL/h/mmHg. The length of dialysis session was between 3.5 and 4 h and was not modified during the study period. When patients switched to post-dilution OL-HDF a minimum of 18 L/session of replacement volume was requested. Both OL-HDF and HD were performed with ultrapure dialysis fluids defined by colony forming unit levels less than 0.1 and endotoxins less than 0.03 UE/mL. All participants (aged over 18) were recruited at the hospital Fundació Puigvert and provided written informed consent and blood samples were collected under protocols approved by the Ethics Committee of the Puigvert Foundation. Standard blood analysis was also carried out at the Puigvert Foundation, including the determination of relevant parameters such as calcium, phosphorus, glucose, cholesterol, triglycerides, albumin and hemoglobin, ferritin, iron, transferrin saturation, parathyroid hormone and C-reactive protein. Blood samples were collected simultaneously for comet and micronucleus tests, previously published [18], and sent to the Universitat Autònoma de Barcelona for its processing.

2.2. Comet assay

DNA breaks present in peripheral blood lymphocytes were measured using the comet assay. The assay was performed following the standard protocol previously described [19,20] with minor modifications. Briefly, isolated lymphocytes from 2 mL of blood from each patient were cryopreserved in 500 μL with 90% serum and 10% DMSO, until use. Gelbond® films (with 48 agarose drops, 6 drops per individual) were used instead of microscopic slides as a support for the agarose gel. The use of hydrophilic films facilitates the rapid processing of numerous samples, increasing the efficiency of the alkaline comet technique, without sacrificing the reliability or sensitivity of the assay [21,22]. Samples were immediately processed after their arrival to the laboratory. A total of one hundred randomly selected cells from 8 replicas (16–17 nuclei per replica), were analyzed per patient. Per cent tail DNA was used as a measure of DNA damage, recommended as the best descriptor for DNA breaks frequencies [23,24]. Komet version 5.5 software was used.

2.3. Detection of oxidatively damaged DNA

The comet assay was performed following the standard protocol previously described [13] with minor modifications. Formamidopyrimidine DNA-glycosylase (FPG) was used as lesion-specific repair enzyme to detect oxidatively damaged DNA, as recommended by the European Standards Committee on Oxidative DNA Damage (ESCODD) [25]. FPG-modified comet converts oxidized purines, ring-opened purines and also some alkylation damage in single strand breaks. The FPG cellular extract was produced in our laboratory, from transformed *E. coli* and was used at a final concentration of 8.9 ng/μL for the treatment. Net oxidatively damaged DNA values were calculated by subtracting the damage scored in the samples incubated with buffer from those incubated with FPG. Positive and negative controls were included on each FPG experiment to assure the procedure. For oxidatively damaged DNA values, positive controls had an average of 47.37 ± 6.01. As previously mentioned, 100 randomly selected cells were scored for each patient.

2.4. Trolox equivalent antioxidant capacity (TEAC) assay

The antioxidant capacity of the plasma was measured by using the TEAC assay as we have already described [18]. Venous blood samples collected in EDTA tubes were centrifuged at 170*g* during 5 min to obtain plasma that was stored at −80 °C. Ten μL of plasma or Trolox standard reacted with 6.20 μM myoglobin solution (20 μL), 183 μM ABTS solution (150 μL) and 10 mM H_2O_2 (25 μL) on a microplate. Reaction was followed at 405 nm with