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The effects of 1-year treatment with a haemodiafiltration with on-line regeneration of ultrafiltrate (HFR) dialysis on biomarkers of oxidative stress in patients with chronic renal failure

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Abstract In the last few years haemodiafiltration with on-line regeneration of ultrafiltrate (HFR) has been shown to have a positive impact on inflammation and oxidative stress biomarkers, but its effect on antioxidant levels and on oxidative damage to biomolecules in the long-term is still unknown. This is a randomised clinical study over 12 months involving 40 patients on haemodialysis, comparing the effect of HFR ($n = 25$) dialysis with haemodialysis with polysulfone (HD-PS, $n = 15$) on oxidative stress. Total antioxidant capacity, enzymatic antioxidant [superoxide dismutase (SOD), catalase and glutathione peroxidase], non-enzymatic (GSH) and biomarkers of oxidative stress (TBARs, carbonyl groups and 8-OH-dG) were evaluated. The antioxidant activity decreased in the lymphocytes of patients dialysed with HFR, with a significant decrease in the enzyme SOD. In the oxidative stress biomarkers, an increase was seen in the levels of 8-OH-dG in patients on HD-PS dialysis but not in those treated with HFR. Throughout the year the changes in antioxidant levels and biomarkers of oxidative damage in patients dialysed with HFR were generally more modest and fluctuated less than those dialysed with HD-PS. Our study indicates that, in general, long-term dialysis with HFR does not modified antioxidant parameters or increases the oxidative damage to biomolecules. The HFR showed to be a biocompatible technique for long-term dialysis.

Keywords HFR · Total antioxidant capacity · Antioxidant enzymes · Carbonyl group · TBARs · 8-OH-dG

Introduction

The increase in oxidative stress in dialysed patients seems to be a result of the accumulation of compounds with pro-oxidant capacity due to the activation of reactive oxygen species (ROS) by neutrophils and platelets, or antioxidant depletion due to these being consumed or eliminated during the dialysis process [1–3]. This increased of oxidative stress in these patients with chronic renal failure is a factor contributing to the morbidity of these patients. At present, there is much research in the field of nephrology showing that a lower stress oxidative by the increase of antioxidants or by the decreased of the oxidants is associated with a lower damage oxidative to biomolecules [4, 5].

A classic renal replacement therapy is haemodialysis using high flow polysulfone (PS) membranes that improve the ROS/antioxidant ratio [6–8]. But in the last few years there has been growing interest in the on-line production of reinfusion fluids with the aim of reducing the risks arising from the infusion of volumes of exogenous fluids. haemodiafiltration with on-line regeneration of ultrafiltrate (HFR) is a novel dialysis technique that combines the processes of diffusion, convection and adsorption [9–11]. HFR appears to have a positive impact on inflammation

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and oxidative stress markers [11–13]. Thus, in a preliminary study carried out by our research group [13] it was noted that dialysis with HFR induced less oxidative stress than haemodialysis with PS (HD-PS). Although these results showed less oxidative damage after one dialysis session, it still needed to be determined whether the HFR technique over the long-term induced stable or fluctuating changes not directly associated with the progression of the renal disease.

Therefore, the aim of this study was to examine, for the first time, the effects of HFR technique on the oxidative stress markers throughout one year, and compare them with a group of patients dialysed with HD-PS. We evaluated oxidative stress by measuring the total antioxidant capacity (TAC) of the plasma, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), and reduced glutathione (GSH) levels, as well as biomarkers of oxidative damage to lipids (TBARs), proteins (carbonyl groups) and DNA (8-OH-dG).

Patients and methods

This multicentre study was approved by the Clinic Research Ethics Committee of Hospital General Yague (Burgos, Spain). Patients were selected from different Hospitals in Spain. One group of patients ($n = 25$) was treated with HFR (SG8–Plus-Bellco) and another group ($n = 15$) with haemodialysis with polysulfone (HD-PS) (High-flux, Fresenius HF80, 1.8 m^2). To be included in the study, patients must have received the same mode of dialysis for least 12 weeks before the study. Patients were selected using the following inclusion criteria: ferritin levels <600 ng/ml, diuresis <300 ml/24 h, non-smokers, lack of comorbidity, such as diabetes, hyperparathyroidism, cancer and chronic liver disease.

Blood samples were collected at the beginning of the study (basal) and every 3 months for 1 year. Plasma was separated by centrifugation (3000×*g*, 10 min) and stored at −70°C. The lymphocytes were isolated by Histopaque density gradient centrifugation.

Antioxidant parameters

TAC was determined by the 2,2′-azino-bis-3-etilbenzothiazol-6-sulfonic acid (ABTS) method [14]. The SOD activity was measured according to the method developed by McCord and Fridovich [15]. Catalase activity was assayed using the method described by Clairbone [16] and the GPX activity was determined by the method described by Gunzler and Flohé [17]. The GSH levels were assayed by the glutathione-*S*-transferase method according to Brigelius et al. [18].

Biomarkers of oxidative stress

Thiobarbituric-acid-reactive substances (TBARs) were assayed according to Stacey and Priestly [19]. Protein oxidation was measured by analysing the carbonyl groups formed using the method of Levine and Stadtman [20]. Total protein concentration was evaluated according to the method of Lowry et al. [21] using bovine albumin as a standard.

For the quantification the levels of 8-hydroxy-2-deoxyguanosine (8-OH-dG), the lymphocytes were isolated by the Ficoll method and the isolation and enzymatic digestion of DNA was carried out as previously described [22]. The levels of 8-OHdG in the DNA were measured by HPLC with electrochemical detection under previously described conditions [22, 23].

Statistics analysis

The data sets were compared using analysis of variance (ANOVA) and the least significant differences (LSD) test calculated to a significance level of $\alpha = 0.05$.

Results

The clinical characteristics of HFR and HD-PS patients show that the prevalence of hypertension was high in both groups (87.5% for HFR and 75% for HD-PS). The triglyceride and cholesterol levels were lower in the group of HD-PS patients, but the differences were only significant for the cholesterol levels (142 ± 22 mg/dL vs. 180 ± 34 mg/dL). Although the parathyroid hormone (PTH) values were higher in HFR-patients, the difference not was significant (227 ± 161 pg/mL vs. 148 ± 97 pg/mL).

The plasma TAC, of patients dialysed with HFR and HD-PS was measured by the ABTS method and is shown in Fig. 1**.** A slight decrease in the plasma antioxidant capacity at the end of the study can be seen in both groups, although there is no significant difference at 12 months compared to the baseline time. Figure 2 summarises the levels of antioxidant enzymes in blood and lymphocytes of HFR and HD-PS patients. Significant changes were seen in the enzyme SOD, with a significant decrease in activity from 6 months ($P < 0.05$) for HFR and HD-PS, both in the plasma and lymphocytes. The SOD activity was 1.5 times lower than at baseline time in the blood of patients dialysed with HD-PS. No significant changes were seen in catalase and GPX activities in the blood or lymphocytes throughout the year of the study. A decrease in the GSH levels (Fig. 3) was observed at 6 months in the lymphocytes of patients dialysed with HFR and with HD-PS, but was only significant for the HFR group. The oxidative damage biomarkers,

TBARs, carbonyl groups and modified base 8-OH-dG in plasma and lymphocytes are shown in Fig. 4. No changes were observed in the levels of TBARs and carbonyl groups. However, an increase was observed in 8-OH-dG levels at the end of the study in the group of patients dialysed with the HD-PS technique, although there were no statistically significant differences.

Discussion

Various studies have shown that, in general, there is an increase in the oxidative stress biomarkers [24–26] and a decrease in the antioxidant system [27–30] in patients with chronic renal failure on chronic dialysis. HFR appears to have a positive effect on markers of inflammation and on the biomarkers of oxidative stress [12, 28].

TAC integrates the cumulative effect of all antioxidants present in the plasma and body fluids and may give more relevant biological information compared to that obtained by the measurements of individual parameters [28]. The results indicate that the HFR technique is biocompatible, as shown by the lower and more homogenous TAC levels over the year compared with the group dialysed with HD-PS. As regards the antioxidant enzymes, the SOD enzyme was the most sensitive, with a significant decrease throughout the year in blood and lymphocytes, although this change was less in those dialysed with HFR, and this could be associated to a lower inflammatory response [12]. The slight changes observed in the catalase and GPX enzymes is in agreement with those obtained by other authors who observed significant decreases in antioxidant activity only after 7 years of dialysis treatment [24].

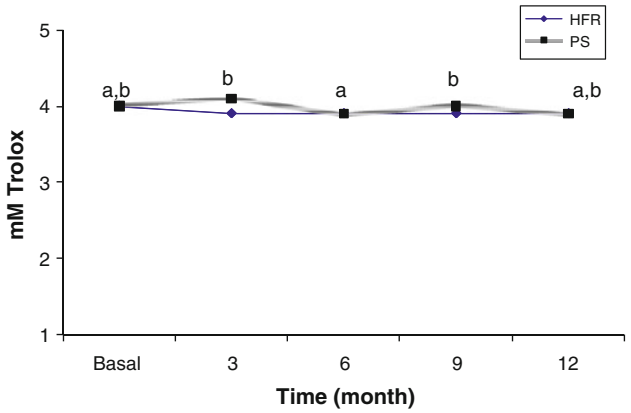

Fig. 1 Total antioxidant capacity in the plasma of patients dialysed with HFR and HD-PS. Values are expressed as mean ± standard deviation. The *letters* indicate significant differences ($P < 0.05$) along the time for patients dialysed with HFR. No statistically significant differences along the time in the patients dialysed with HD-PS

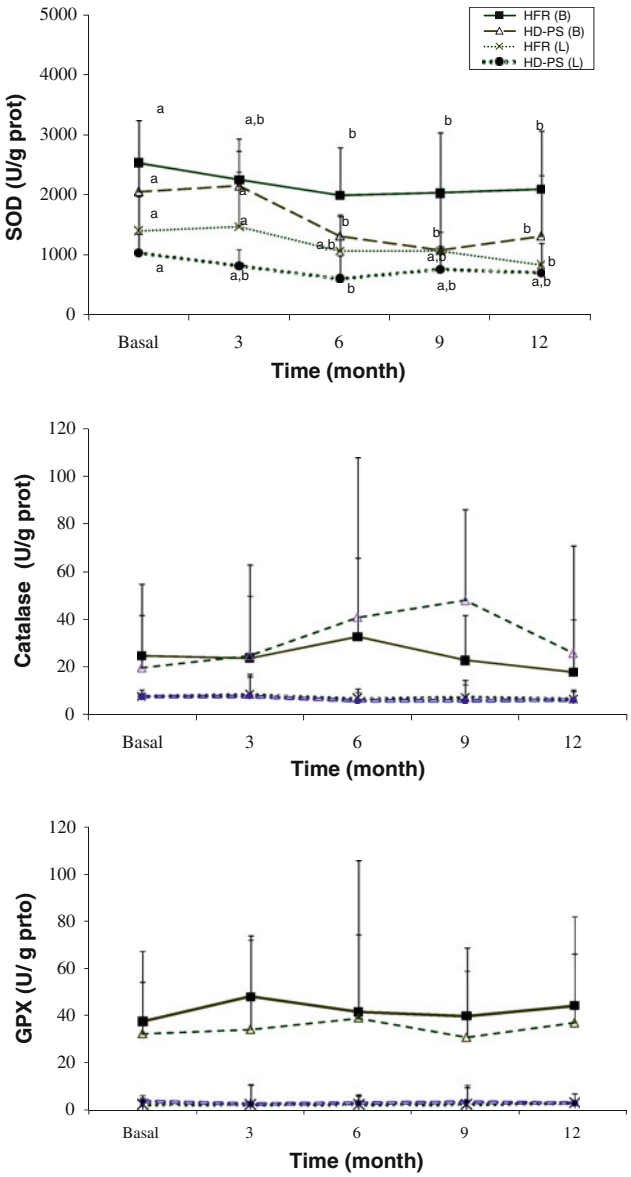

Fig. 2 Enzymatic antioxidant activity in the blood and lymphocytes of patients dialysed with HRFR and HD-PS. *SOD* superoxide dismutase, *GPX* glutathione peroxidase. The activities in blood are expressed as U/g Hb and in lymphocytes as U/g prot. The results are mean ± SD. Different *letters* indicate significant differences ($P < 0.05$) between means within same dialysis group along time

Glutathione is the principal low molecular weight intracellular antioxidant responsible for the redox state in cells and its levels in patients subjected to dialysis are decreased as a result of GSH turnover [31]. Our results show that the behaviour of this tripeptide depends of the medium in which it is studied. No changes were observed in blood, whereas there was a decrease in the lymphocytes, which was significant in patients, dialysed with HFR at 3 months. This could be due to adapting to the dialysis technique.

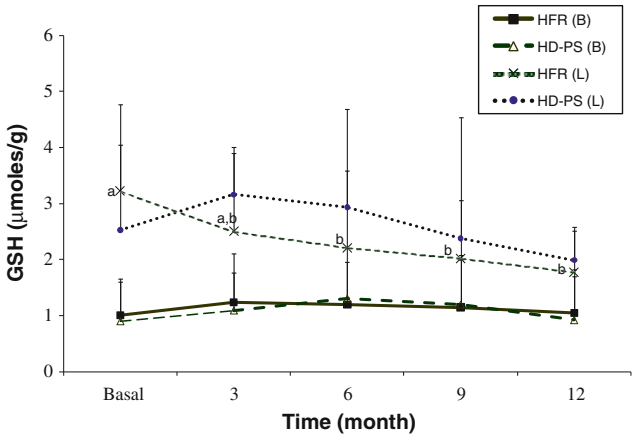

Fig. 3 GSH levels in the blood and lymphocytes of patients dialysed with HFR and HD-PS. The GSH levels in blood are expressed as µmol/g Hb and in lymphocytes as µmol/g prot. The results are mean ± S.D. Different *letters* indicate significant differences ($P < 0.05$) between means within same dialysis group along time

The results of the oxidative stress biomarkers show that dialysis treatment with the HFR technique, like that with HD-PS, does not increase the carbonyl groups or the levels of TBARs significantly. These results are in agreement with those observed by other authors [24, 25, 32–34]. There are few studies that have looked at the influence of long-term dialysis on DNA oxidative damage [35–37]. In the present study we have seen a difference between the two dialysis treatments. Although no changes were seen in the 8-OH-dG levels of patients dialysed with HFR throughout the study, these did increase, although not significantly, in patients dialysed with HD-PS at one year. These results agree with those observed by Satoh et al., who in 2001 [38] observed high levels of 8-oxo-dG in patients dialysed with HD-PS. The authors commented that this could be due to an increase in oxidative stress because they noted that the levels of this modified base decreased when they used a membrane coated with vitamin E.

In conclusion, we suggest that HFR has a higher biocompatibility compared to classic HD-PS dialysis. This is reflected in the changes that were obtained in the analysis oxidative stress biomarkers, which were lower and showed less fluctuation over the year, and perhaps indicates that this technique is resistant to oxidative stress.

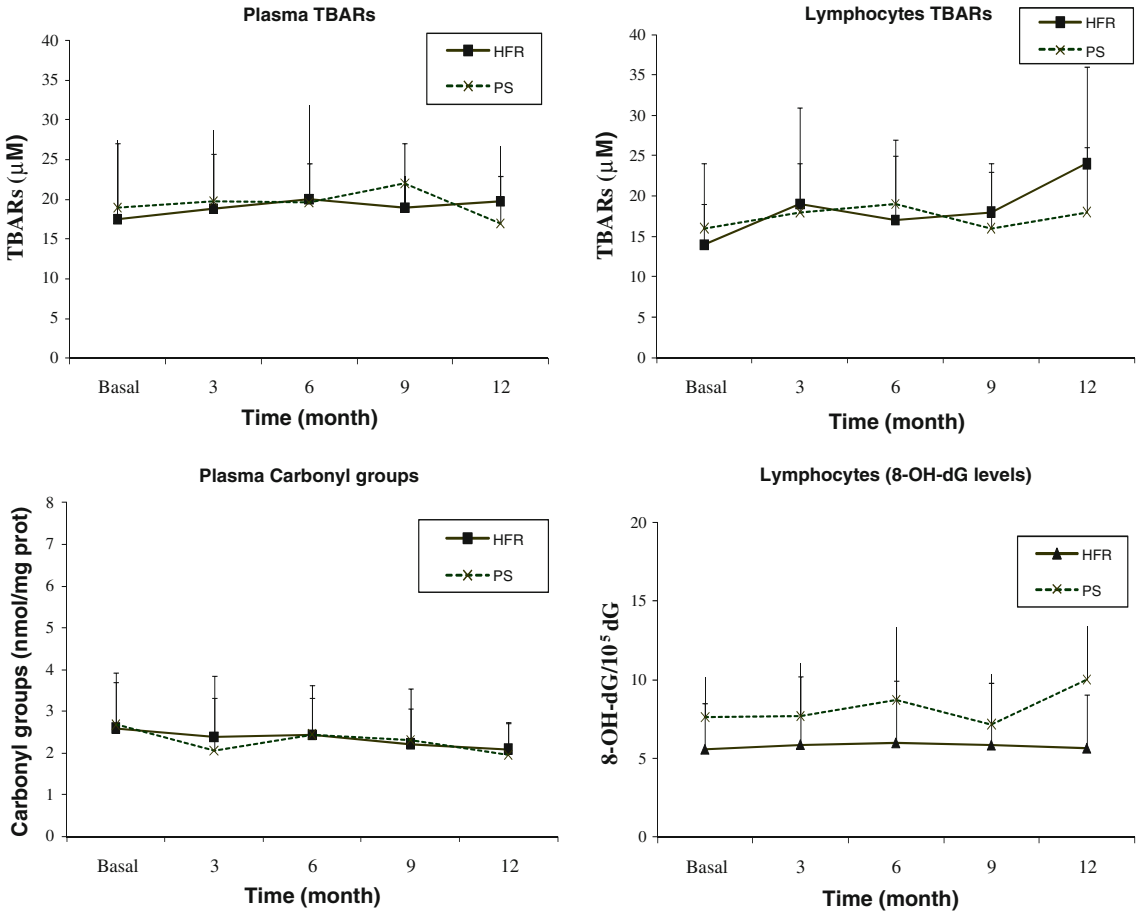

Fig. 4 Levels of biomarkers in plasma TBARs (**a**) and carbonyl groups (**b**) and in lymphocytes TBARs (**c**) and 8-OH-dG levels (**d**) of patients dialysed with HFR and HD-PS. The results are mean ± standard deviation

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