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Mortality and cardiovascular events in online haemodiafiltration (OL-HDF) compared with high-flux dialysis: results from the Turkish OL-HDF Study

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Abstract

Background. Online haemodiafiltration (OL-HDF) is considered to confer clinical benefits over haemodialysis (HD) in terms of solute removal in patients undergoing maintenance HD. The aim of this study was to compare postdilution OL-HDF and high-flux HD in terms of morbidity and mortality.

Methods. In this prospective, randomized, controlled trial, we enrolled 782 patients undergoing thrice-weekly HD and randomly assigned them in a 1:1 ratio to either postdilution OL-HDF or high-flux HD. The mean age of patients was 56.5 ± 13.9 years, time on HD 57.9 ± 44.6 months with a diabetes incidence of 34.7%. The follow-up period was 2 years, with the mean follow-up of 22.7 ± 10.9 months. The primary outcome was a composite of death from any cause and nonfatal cardiovascular events. The major secondary outcomes were cardiovascular and overall mortality, intradialytic complications, hospitalization rate, changes in several laboratory parameters and medications used.

Results. The filtration volume in OL-HDF was 17.2 ± 1.3 L. Primary outcome was not different between the groups (event-free survival of 77.6% in OL-HDF versus 74.8% in the high-flux group, P = 0.28), as well as cardiovascular and overall survival, hospitalization rate and number of hypotensive episodes. In a *post hoc* analysis, the subgroup of OL-HDF patients treated with a median substitution volume >17.4 L per session (high-efficiency OL-HDF, $n = 195$) had better cardiovascular (P = 0.002) and overall survival (P = 0.03) compared with the high-flux HD group. In adjusted Cox-regression analysis, treatment with high-efficiency OL-HDF was associated with a 46% risk reduction for overall mortality {RR = 0.54 [95% confidence interval (95% CI) 0.31–0.93], P = 0.02} and a 71% risk reduction for cardiovascular mortality [RR = 0.29 (95% CI 0.12–0.65), P = 0.003] compared with high-flux HD.

Conclusions. The composite of all-cause mortality and nonfatal cardiovascular event rate was not different in the OL-HDF and in the high-flux HD groups. In a *post hoc* analysis, OL-HDF treatment with substitution volumes over 17.4 L was associated with better cardiovascular and overall survival.

Keywords: high-flux haemodialysis; online haemodiafiltration; outcome

Introduction

Cardiovascular diseases (CVD) are common in patients on conventional haemodialysis (HD), performed thrice weekly. Despite refinements of dialysis therapy, both overall and cardiovascular mortality rates in patients treated with conventional HD are much higher than those seen in the nonuraemic population [1]. The increased risk of mortality of dialysis patients can in part be explained by an ageing population and increased prevalence of comorbid factors such as diabetes and hypertension. Moreover, a number of risk factors unique to uraemia itself, including accumulation of uraemic toxins, chronic inflammatory state and mineral metabolism disorders may contribute to the high prevalence of CVD. In particular, retention of middle or large middle-sized molecules is considered to impact the pathogenesis of CVD [2]. It is therefore reasonable to assume that dialysis treatment modalities that increase the removal of middle molecules may reduce the incidence of CVD and thereby contribute towards improved patient survival.

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Removal of larger uraemic retention solutes, commonly referred to as uraemic toxins, is limited in conventional HD therapies. While usage of high-flux membranes enables the removal of larger uraemic toxins and has been related to better outcomes in retrospective studies [3, 4], two prospective randomized trials (HEMO and MPO studies) failed to demonstrate a survival benefit, except in subgroup analyses [5, 6]. In the primary analysis of the HEMO study, high-flux HD was associated with an 8% nonsignificant reduction of mortality compared with low-flux HD. However, a secondary analysis revealed significant survival benefit with high-flux in patients who were on dialysis for more than 3.7 years [5]. The MPO study found that high-flux HD showed greater survival compared with the low-flux HD in high-risk patients with serum albumin <4 g/dL or in a *post hoc* analysis in patients with diabetes [6]. Online haemodiafiltration (OL-HDF), which combines diffusive and convective transport, is superior to conventional HD in terms of clearance of small solutes, such as urea and middle molecules, like β-2 microglobulin. It has been suggested that OL-HDF (utilizing biocompatible high-flux membranes and ultrapure dialysis fluids) may improve clinical outcomes through enhanced small, middle and larger protein-bound uraemic solute clearance [7–9], better intradialytic haemodynamic stability, reduced inflammation, anaemia correction and improved phosphate control when compared with conventional HD [10–12]. The Dialysis Outcomes and Practice Patterns Study (DOPPS) found that the high-efficiency OL-HDF treatment (high-volume substitution 15–25 L) was associated with better survival compared with low-flux HD [12]. While an association with better survival of OL-HDF has also been reported in the prospective and observational RISCAVID study [13], other prospective and randomized studies involving small patient numbers have not been able to demonstrate any survival advantage over the high-flux HD treatment modality [14, 15]. Recently, a retrospective analysis showed that treatment predominantly with OL-HDF was associated with better survival compared with high-flux HD in incident patients [16].

The aim of this prospective and randomized clinical trial was to compare the effects of postdilution OL-HDF and conventional high-flux HD on a primary composite endpoint of all-cause mortality and nonfatal cardiovascular events. Clinical secondary endpoints included intradialytic complications, medication requirements, changes in blood pressure (BP), hospitalization rate and laboratory parameters.

Materials and methods

Study design

The 'Comparison of Post-dilution Online Haemodiafiltration and Haemodialysis (TURKISH HDF STUDY)' was an open-label, prospective, multicentre, randomized trial (Clinical Trials ID, NCT00411177). Primary and secondary outcomes were evaluated during a minimum 24-month follow-up period. Owing to an initial slow recruitment of patients for the study, the follow-up reached a maximum observation period of 39 months.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki; all patients provided written informed consent. The study was also performed in compliance with the Good Clinical Practice Guidelines. The local ethics committee of Ege University Izmir, Turkey, approved the study protocol. Physicians at the dialysis clinics, in accordance with national health authority regulations, assessed all individual treatment sessions. An independent institution (Data Management Service) at Ege University collated and managed the data of the trial.

Treatment characteristics

The intended dialysis treatment duration for both modality arms of the trial was 240 min with a blood flow rate between 250 and 400 mL/min. The dialysate flow rate was kept at 500 mL/min in both groups. The same high-flux dialysers, either FX60 or FX80 (Polysulfone-based Helixone Membrane, Fresenius Medical Care, Bad Homburg, Germany) were used during the entire study period. Dialysate composition was the same in >90% of subjects in both arms of the study (Na 138 mmol/L, K 2.0 mmol/L, Ca 1.5 mmol/L, Mg 0.5 mmol/L, Cl 109 mmol/L, HCO_3 32 mmol/L, acetate 3 mmol/l, glucose 5.5 mmol/L). Sodium modelling was not applied. Unfractionated heparin (50 U/kg bolus followed by 1000 U/h infusion) was used for anticoagulation. Dialyser reuse was not permitted. The same water production systems were used in all study centres. Standard dialysate was utilized in the high-flux HD group.

OL-HDF procedure was performed in the postdilution mode under strict safety operational procedures. Fresenius 4008S dialysis machines, incorporating the ONLINE*plus* (Fresenius Medical Care, Bad Homburg, Germany) system were used. This system consists of two ultrafilters (DIASAFE*plus*); the first one is placed after the proportioning system and the second is positioned before the substitution port. Ultrafilters installed on the haemodiafiltration (HDF) machine were replaced after 100 treatments or 12 weeks of use, whichever came first. Dialysate in the high-flux HD group and infusate in the OL-HDF group were regularly assessed for colony-forming units and endotoxin levels before change of ultrafilters. In the OL-HDF mode, the filtration rates were adjusted to be between 25 and 30% of the achieved blood flow rate and substitution volume was targeted to be above 15 L per session [12]. The electrolyte composition of the infusate was the same as the composition of the dialysis fluid. The effective substitution volume (without the ultrafiltrate volume) used in analyses was calculated as mean of substitution volumes recorded in all sessions during follow-up, in which HDF treatment was performed (94.4%).

Patient selection

The 782 patients who agreed to take part in the study were enrolled between January 2007 and March 2008 in 10 HD centres operated by Fresenius Medical Care in south and southeast Turkey, and then randomized centrally in a 1:1 ratio to either the OL-HDF or high-flux HD treatment arm of the study (Figure 1). Patient eligibility criteria were >18 years, maintenance bicarbonate HD scheduled thrice weekly for a total of 12 h/week, have achieved mean single-pool Kt/V >1.2 and willingness to participate in the study with signed informed consent. Exclusion criteria were scheduled for living donor renal transplantation, serious life-limiting comorbid situations, namely active malignancy, active infection, end-stage cardiac, pulmonary or hepatic disease, requirement for HD more than three times per week due to comorbid conditions, temporary catheter as a vascular access, insufficient vascular access (blood flow rate lower than 250 mL/min), presence of urine output more than 250 mL/day, pregnancy or nursing mothers, mental incompetence.

Study endpoints

The composite of all-cause mortality and first nonfatal cardiovascular event including myocardial infarction, stroke, coronary revascularization and unstable angina pectoris requiring hospitalization was defined as primary outcome. The classification of myocardial infarction and unstable angina pectoris requiring hospitalization were based on clinical symptoms and electrocardiography findings as well as on markers of cardiac muscle damage, including creatinine kinase-MB. Definition of stroke was based on neurological symptoms caused by an ischemic or haemorrhagic event detected in radiological examinations.

Causes of death were categorized as either cardiovascular or noncardiovascular. The main secondary outcome was cardiovascular mortality; all deaths, including sudden death, were defined as cardiovascular in nature unless a noncardiovascular cause could be identified. Additional secondary outcome parameters were hospitalization rate, intradialytic complications including hypotension and changes in the following parameters: BP, postdialysis body weight, heamatocrit, erythropoietin dose and resistance to erythropoeitin. Moreover, the levels of phosphorus, albumin, total cholesterol, high-density lipoprotein (HDL) cholesterol,

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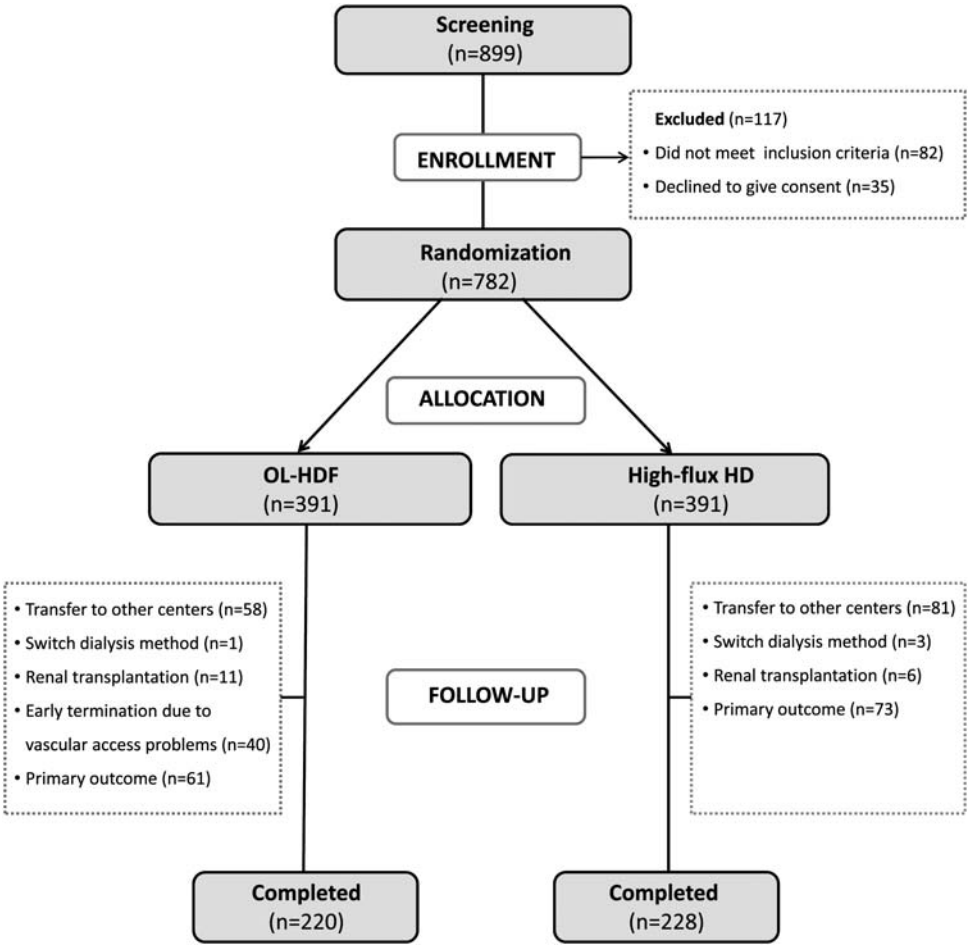

Fig. 1. Flow chart of study participation.

low-density lipoprotein (LDL) cholesterol, triglyceride, high-sensitivity C-reactive protein (hs-CRP), β-2 microglobulin and required medications were examined.

Methods

During the study period, laboratory parameters were studied monthly, except ferritin, transferrin saturation, lipid parameters and hs-CRP, which were measured every 3 months by using standard automated techniques (Architect C 8000 auto-analyser and Axsym third generation immunoassay system, Abbott, IL). Serum-intact parathyroid hormone (PTH; Elecsys 2010, Roche Diagnostics) and β-2 microglobulin (Axsym third generation immunoassay system, Abbott, IL) were measured every 6 months. Blood samples were obtained under fasting conditions immediately before the patients' scheduled dialysis sessions and either studied within 2 h following centrifugation or sera samples were stored at −70°C until measurement. All analyses were carried out at a central laboratory (DIALAB) registered to external quality control programs.

The quality of water used for dialysis fluid was assessed by taking microbial counts from dialysis and infusion fluids. All samples were taken and analysed according to a standard protocol [17]. Determination of microbial counts were carried out by standard methods agar and expressed as colony-forming units per millilitre dialysate (CFU/mL). Endotoxin was assessed with the limulus amebocyte lysate (LAL) assay (Coatest Endotoxin Chromogenix, Mölndal, Sweden) and expressed as LAL activity in EU/mL. In the OL-HDF group, targets for infusate purity were microbial count $<10^{-6}$ CFU/mL and endotoxin levels <0.03 EU/mL. In the high-flux HD, dialysate purity targets were a total of dialysate microbial count lower than 200 CFU/mL and endotoxin concentration of lower than 2 EU/mL [17].

For postdialysis body weight, interdialytic weight gain (IDWG) and predialysis BP, the mean of all values recorded in all sessions of each month were calculated. BP measurements were made manually using an Erka sphygmomanometer after a 5-min rest just before the dialysis session. Intradialytic hypotension was defined as symptomatic BP drop (>30 mmHg in systolic BP) requiring saline infusion. During the study period, medication data were recorded monthly. The majority of the patients receiving erythropoiesis-stimulating agents (92.2%) have used recombinant human erythropoietin (r-HuEPO), the remaining ones darbepoetin alfa. Darbepoetin alfa dose was converted to r-HuEPO dose by multiplying with 200, with monthly iron administration based on ferritin and TSAT measurements.

Sample size estimation

Sample size was estimated according to the following assumptions: 2-year follow-up, the annual rate of primary endpoint in thrice weekly conventional high-flux HD of 20% and a two-sided type I error of 5%, an 80% power to detect a decrease of 35% in the annual rate of primary endpoint in the patients treated with OL-HDF in comparison to HD. The assumption for the 35% risk reduction was based on results of the DOPPS by Canaud *et al.* [12]. We estimated the annual rate of the primary endpoint to be 20% on the basis of an annual mortality rate of 12% in the clinics involved in the study and a nonfatal CV event rate of 7% occurring in two clinics in the preceding year. The required sample size was thus estimated to be 780 patients, assuming an annual dropout rate of 25%.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Baseline values of the two groups were compared using the two-sample Student's *t*-tests

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or chi-square test for categorical data, as appropriate. The data of the patients who were transferred to another treatment modality or to other dialysis centres were considered for analyses until the time of premature discontinuation. Survival analysis was performed using the Kaplan–Meier method, testing for statistical significance using the log-rank test. The sensitivity of univariate analysis results was checked by comparing the analyses performed with study groups excluding patients who dropped out. For independent predictors associated with primary outcome, adjusted forward stepwise Cox regression analysis was performed including those variables statistically significant in the univariate analysis (age, diabetes, CVD history, vascular access, serum creatinin, albumin and hs-CRP) and those variables that could have an effect on primary outcome (gender and time on HD).

Post hoc analyses

On the basis of the findings of Canaud *et al.* [12], we targeted to achieve a substitution volume above 15 L per session in the OL-HDF arm of the study. However, as a *post hoc* analysis, to examine the impact of substitution volumes even higher than this minimal target, we categorized patients on OL-HDF into two subgroups, one above and the other below the median of time-averaged substitution volume provided to the patient population during the study. The impact of delivered dose of OL-HDF quantified in terms of volume of substitution fluid was analysed by a Cox-regression model including those variables statistically significant ($P \leq 0.1$) in univariate analysis among the groups at baseline (diabetes, blood flow rate, serum albumin, phosphate, haemoglobin, equilibrated Kt/V (eKt/V) and IDWG) and those variables that could have an effect on primary outcome (age, gender, time on HD, CVD and vascular access). Other subgroup analyses including diabetic patients, the patients with CVD history and serum albumin below 4 g/dL were also done *post hoc*.

Statistical significance was defined as $P < 0.05$. All analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics and laboratory parameters of the patients randomized into the two arms of the study were similar (Tables 1 and 2). In the randomized patient population, the mean age was 56.5 ± 13.9 years and 41.1% were female. The prevalence of diabetes mellitus and CVD history were 34.7 and 26.4%, respectively. At the start of the study, the patients were on dialysis for a mean duration of 57.9 ± 44.6 months. The majority of the patients (95%) had native arteriovenous (AV) fistulae as vascular access. At baseline, BP was adequately controlled in 84% of the patients (systolic BP ≤ 140 and diastolic BP ≤ 90 mmHg) with 13% patients receiving antihypertensive medications. Hypoalbuminaemia (<4 g/dL) was present in 58% of the patients. Seventy-seven percent of the patients had hs-CRP levels >0.5 mg/dL. The majority of the patients had serum phosphate levels below 5.5 mg/dL (72.2%); only 16.4% of the patients had a calcium-phosphate product >55 mg^2/dL^2.

Primary outcome

The mean follow-up period was similar in the two treatment groups: 22.8 ± 10.6 months (range 1.3–38.5 months) in the OL-HDF group and 22.6 ± 11.2 months (range 1.4–36.5 months) in the HD group ($P = 0.81$). During the follow-up, 160 patients left the study for reasons other than death: 17 received renal transplantation (11 in the OL-HDF group and 6 in the HD group), 143 switched to other modes of dialysis (1 in the OL-HDF group and 3 in the HD group) or were transferred to nonparticipating centers (58 in the OL-HDF group and 81 in the HD group). Forty patients in the OL-HDF group terminated the study early due to vascular access problems, mainly the insufficient blood flow rate. Baseline demographical, clinical and laboratory parameters were not different between the patients who remained in the study from the OL-HDF and from the HD group (data not shown).

Kaplan–Meier survival analysis for the primary endpoint comparing the treatment groups is shown in Figure 2. The

Table 1. Baseline characteristics of the study population

	All patients ($n = 782$)	Online HDF ($n = 391$)	High-flux HD ($n = 391$)	P
Age (years)	56.5 ± 13.9	56.4 ± 13.0	56.5 ± 14.9	0.97
Gender (female, %)	41.1	40.4	41.9	0.66
Etiology of ESRD (%)				
Unknown	36.7	39.8	34.5	0.68
Diabetic nephropathy	30.1	29.6	31.9	0.72
Hypertension	10.6	11.5	9.4	0.67
Chronic glomerulonephritis	3.5	4.3	2.8	0.74
Others	19.1	14.8	21.4	0.36
Diabetes mellitus (%)	34.7	36.3	33.2	0.36
Dialysis duration (months)	57.9 ± 44.6	57.1 ± 43.2	58.7 ± 46.1	0.60
Vascular access (% AV fistula)	95.5	95.7	95.4	0.86
Blood flow rate (mL/min)	294 ± 45	294 ± 46	294 ± 44	0.94
Smoking (%)	24.9	23.8	26.0	0.52
Cardiovascular disease history (%)	26.4	27.2	25.7	0.66
Body mass index (kg/m^2)	24.8 ± 4.8	24.9 ± 4.9	24.8 ± 4.6	0.65
Postdialytic body weight (kg)	67.9 ± 13.4	67.9 ± 13.5	67.9 ± 13.4	0.99
Systolic blood pressure (mmHg)	128 ± 15	128 ± 15	127 ± 16	0.78
Diastolic blood pressure (mmHg)	78 ± 8	78 ± 7	78 ± 8	0.64
Interdialytic weight gain (% body weight)	3.5 ± 1.7	3.5 ± 1.5	3.4 ± 1.8	0.70
Antihypertensive medication (%)	13.6	13.1	14.2	0.82
Phosphate binder use (%)	83.1	82.4	83.9	0.76
Intravenous iron use (%)	57.7	58.1	57.3	0.82
Erythropoietin use (%)	57.3	56.2	58.4	0.78
Erythropoietin resistance index (U/week per kg g/dL)	3.21 ± 3.10	3.18 ± 3.12	3.24 ± 2.89	0.92
Vitamin D use (%)	21.9	22.1	21.7	0.80

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Table 2. Baseline laboratory parameters between the patients treated with OL-HDF and high-flux HD

	Online HDF ($n = 391$)	High-flux HD ($n = 391$)	P
Urea (mg/dL)	136 ± 34	134 ± 35	0.53
Creatinine (mg/dL)	8.0 ± 1.9	8.0 ± 2.3	0.84
Sodium (mEq/L)	136 ± 3	136 ± 3	0.84
Potassium (mEq/L)	5.11 ± 0.75	5.08 ± 0.797	0.60
Urea reduction rate (%)	74.9 ± 6.7	74.5 ± 6.3	0.46
eKt/V	1.44 ± 0.27	1.42 ± 0.25	0.29
Calcium (mg/dL)	8.66 ± 0.74	8.69 ± 0.67	0.50
Phosphate (mg/dL)	4.90 ± 1.42	4.88 ± 1.48	0.88
Ca-P product (mg^2/dL^2)	42.5 ± 13.3	42.6 ± 13.4	0.91
Parathyroid hormone (pg/mL)	370 ± 324	359 ± 328	0.66
Albumin (g/dL)	3.83 ± 0.35	3.85 ± 0.38	0.46
Total cholesterol (mg/dL)	173 ± 41	174 ± 43	0.61
Triglyceride (mg/dL)	179 ± 119	184 ± 109	0.59
HDL cholesterol (mg/dL)	43.9 ± 12.4	44.3 ± 12.0	0.66
LDL cholesterol (mg/dL)	93.1 ± 32.4	92.7 ± 31.6	0.87
Haemoglobin (g/dL)	11.4 ± 1.52	11.4 ± 1.44	0.85
Ferritin (ng/mL)	846 ± 644	816 ± 654	0.55
Transferrin saturation (%)	28.0	28.4	0.76
Bicarbonate (mEq/L)	22.7 ± 2.6	22.6 ± 2.5	0.73
Hs-CRP (mg/dL)	1.72 ± 2.38	1.71 ± 2.36	0.93
β-2 MG (mg/L)	26.5 ± 7.9	26.1 ± 9.7	0.57

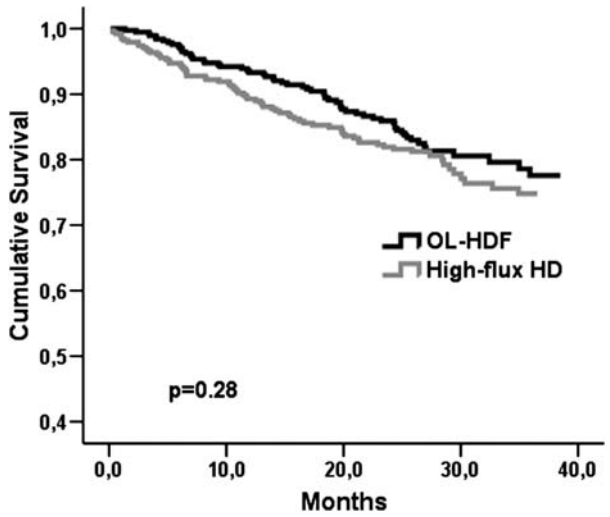

Fig. 2. Composite event-free survival in patients treated with OL-HDF and high-flux HD.

event-free survival rates after 36 months of follow-up with respect to primary outcome were 77.6% in OL-HDF and 74.8% in the HD group, P = 0.28, (8.19 events per 100-patient years in the OL-HDF group and 9.89 events per 100-patient years in the HD group). In crude Cox-regression analysis, the relative risk for composite outcome of OL-HDF treatment was 18% lower compared with HD treatment [95% confidence interval (95% CI) 0.59–1.16, P = 0.28] but failed to reach statistical significance. The patients who dropped out of the study were younger, more likely to be male, had shorter time on HD and higher serum creatinine at baseline compared with the patients who remained in the study (data not shown). Additional analysis using the study group excluding patients who did not complete the whole study period yielded results very similar to the primary analysis (HR = 0.81, 95% CI 0.58–1.14, P = 0.24).

Table 3. Causes of mortality in the studied population during the follow-up period

	All patients ($n = 782$)	OL-HDF ($n = 391$)	High-flux HD ($n = 391$)
Overall mortality (n, %)	117 (15.0)	52 (13.3)	65 (16.6)
Cardiovascular mortality (n, %)	76 (9.7)	32 (8.1)	44 (11.2)
Fatal myocardial infarction (n)	17	6	11
Fatal stroke (n)	15	7	8
Fatal arrhythmia (n)	4	1	3
Sudden death (n)	22	10	12
Congestive heart failure (n)	18	8	10
Noncardiovascular mortality (n, %)	41 (5.2)	20 (5.1)	21 (5.3)
Infection-related (n)	27	14	13
Gastrointestinal haemorrhage (n)	6	3	3
Pulmonary embolism (n)	1	1	–
Malignancy (n)	3	1	2
Hepatic failure (n)	1	–	1
Suicide (n)	1	–	1
Respiratory failure (n)	2	1	1

There were 117 (15.0%) deaths, 52 in the OL-HDF group (13.3%) and 65 in the HD group (16.6%), 76 from cardiovascular causes (32 in the OL-HDF group and 44 in the HD group). The causes of death were shown in Table 3. In multivariate Cox regression analysis, age [hazard ratio (HR) = 1.04, 95% CI 1.02–1.06, P < 0.001], presence of diabetes (HR = 2.28, 95% CI 1.55–3.37, P < 0.001) and AV fistula (HR = 0.41, 95% CI 0.20–0.85, P = 0.01) were independent predictors for primary outcome.

Secondary outcomes

The overall mortality rate was 21% lower in the OL-HDF group (P = 0.21), not reaching statistical significance

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(HR = 0.79, 95% CI 0.55–1.14). The cardiovascular mortality rate was 28% lower in the OL-HDF group compared with the HD group (HR = 0.72, 95% CI 0.45–1.13, P = 0.15).

There were no significant differences in time-averaged postdialysis body weight (68.1 ± 13.7 kg in OL-HDF and 67.4 ± 13.2 kg in the HD group, P = 0.46) and body mass index (25.0 ± 4.9 kg/m^2 in the OL-HDF group and 24.7 ± 4.5 kg/m^2 in the HD group) between the groups. The mean systolic BP level was slightly higher in the OL-HDF group than in the HD group (129 ± 13 and 126 ± 13 mmHg, P = 0.001) despite similar diastolic BP levels (77 ± 6 versus 77 ± 7 mmHg, P = 0.07).

The mean IDWG was higher in OL-HDF arm than in the HD group during the course of the study (3.5 ± 1.9% in HDF versus 3.2 ± 1.5% in HD, P = 0.01). The proportion of patients with prescribed antihypertensive medication was similar between the groups during the follow-up (11.1% in the OL-HDF and 11.7% in the HD group, P = 0.66).

The results of mean biochemical parameters are shown in Table 4. The mean hs-CRP levels were similar between the groups as were plasma calcium, phosphate, Ca-P product and PTH levels. Predialysis plasma β-2 microglobulin levels remained stable in both groups with no difference at the end of the study (Delta β-2 microglobulin –0.67 ± 9.57 mg/L in the OL-HDF and –0.59 ± 9.02 mg/L in the HD group, P = 0.94). Despite comparable serum haemoglobin levels, the mean prescribed erythropoietin dosage was significantly lower in the OL-HDF group than in the HD group (2282 ± 2121 versus 2852 ± 2702 U/week, respectively, P = 0.001). The mean prescribed intravenous iron dosage (17 ± 19 mg/week in the OL-HDF group and 18 ± 25 mg/week in the HD group, P = 0.35). During the follow-up, the mean transferrin saturation (27.7 versus 27.5%, P = 0.82) and ferritin levels (819 ± 506 ng/mL versus 809 ± 580 ng/mL, P = 0.80) were similar in the OL-HDF and high-flux HD.

The incidence of hospitalization was similar between the two treatment arms (20.4 per 100 patient-years in the OL-HDF versus 18.6 per 100 patient-years in the HD group, P = 0.44). The frequency of intradialytic hypotensive episodes was not different between the groups during the follow-up (77.7 per 1000 sessions for the OL-HDF group and 81.0 per 1000 sessions in the HD group, P = 0.64).

During the follow-up, the mean substitution volume in the OL-HDF group was 17.2 ± 1.3 L (13.5–20.0 L); 96.7% of the patients were treated with >15 L replacement volume per session. The mean duration of the dialysis sessions was 236 ± 6 min in the OL-HDF and 236 ± 11 min in the HD groups (P = 0.75). The mean blood flow rate in the OL-HDF group was 318 ± 27 mL/min, significantly higher than in the HD group (303 ± 32 mL/min) during the study (P < 0.001). The mean prescribed heparin dose was significantly higher in the OL-HDF group than in the HD group (4977 ± 1598 U versus 4010 ± 1361 U, respectively, P < 0.001). The mean eKt/V during follow-up was 1.44 ± 0.19 in the OL-HDF group, significantly higher (P < 0.001) than in the HD group (1.33 ± 0.19) (Table 4). During the follow-up, microbial count was in the target range in both treatment groups. Endotoxin concentration was undetectable in the replacement fluid in the periodical measurements during the study period. In the high-flux HD arm, endotoxin concentration was below 1 EU/mL in all measurements (mean 0.14 ± 0.04 EU/mL, range from 0.04 to 0.82 EU/mL).

Subgroup analysis

We analysed the impact of OL-HDF on the main outcome parameters in the high-risk populations separately using the following criteria: diabetes, CVD history and low serum albumin levels. In the 272 diabetic subjects, a 26%

Table 4. Time-averaged biochemical parameters in the treatment arms

	Online HDF (n = 391)	High-flux HD (n = 391)	P
Urea (mg/dL)	124 ± 21	129 ± 23	0.002
Creatinine (mg/dL)	8.0 ± 1.8	8.2 ± 2.1	0.11
Urea reduction rate (%)	75.2 ± 4.7	73.2 ± 5.3	<0.001
eKt/V	1.44 ± 0.19	1.33 ± 0.19	<0.001
Sodium (mEq/L)	136 ± 2	135 ± 2	0.13
Potassium (mEq/L)	5.2 ± 0.5	5.2 ± 0.5	0.87
Calcium (mg/dL)	8.94 ± 0.61	8.92 ± 0.56	0.55
Phosphate (mg/dL)	4.66 ± 1.00	4.72 ± 1.01	0.38
Ca-P product (mg^2/dL^2)	41.7 ± 9.7	42.2 ± 9.6	0.52
Parathyroid hormone (pg/mL)	386 ± 291	371 ± 292	0.54
Albumin (g/dL)	3.93 ± 0.24	3.99 ± 0.27	0.001
Total cholesterol (mg/dL)	170 ± 37	170 ± 39	0.91
Triglyceride (mg/dL)	173 ± 97	191 ± 107	0.01
HDL cholesterol (mg/dL)	37 ± 11	34 ± 9	0.007
LDL cholesterol (mg/dL)	99 ± 29	97 ± 30	0.52
Haemoglobin (g/dL)	11.5 ± 1.2	11.5 ± 1.2	0.86
Ferritin (ng/mL)	819 ± 506	809 ± 580	0.80
Transferrin saturation (%)	27.7	27.5	0.82
Erythropoietin dose (U/week)	2282 ± 2121	2852 ± 2706	0.001
Erythropoietin resistance index (U/week per kg per g/dL)	3.19 ± 3.15	3.90 ± 3.72	0.004
Bicarbonate (mEq/L)	22.5 ± 1.79	21.9 ± 1.96	<0.001
Hs-CRP (mg/dL)	1.48 ± 1.63	1.47 ± 1.52	0.88
β-2 Microglobulin (mg/L)	27.1 ± 6.4	27.2 ± 6.8	0.82
Delta β-2 microglobulin (mg/L)	−0.67 ± 9.5	−0.59 ± 9.02	0.94

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lower relative risk (RR: 0.74, 95% CI 0.47–1.18, P = 0.21) for the composite outcome was detected in the OL-HDF group compared with the HD group. Trends of a beneficial effect of OL-HDF were also observed for overall mortality (P = 0.20) and cardiovascular mortality (P = 0.13); the primary event-free survival here was 66.8% in the OL-HDF and 63.4% in the high-flux HD group after 36 months of follow-up. In patients with CVD prior to the start of the study (n = 182), the primary outcome was not significantly different between the groups during the study period (51.9% in the OL-HDF group and 64.9% in the HD group, P = 0.67); overall (P = 0.87) and cardiovascular mortality (P = 0.55) were also similar in the two groups.

In patients with serum albumin < 4 g/dL at baseline (n = 458), the risk of primary outcome was 0.81 (95% CI 0.55–1.22, P = 0.31) in the OL-HDF group compared with the HD group. Overall (75.7 versus 71.2%, P = 0.17) and cardiovascular survival (85.3 versus 80.2%, P = 0.09) was slightly higher in patients treated with OL-HDF than in the HD group.

Impact of higher infusion volumes in OL-HDF on clinical outcomes

The median value of substitution volume in the OL-HDF group was 17.4 L. Stratifying patients according to this threshold, those in the low-efficiency OL-HDF group (≤17.4 L) were more likely to have diabetes, had lower albumin levels but higher haemoglobin levels together with lower erythropoietin dosage at baseline compared with the high-efficiency OL-HDF (>17.4 L) and high-flux HD groups (Table 5). The mean prescribed intravenous iron dosage was not different among the groups. Baseline serum phosphate levels were higher in the low-efficiency OL-HDF group than in the high-efficiency OL-HDF group.

In Kaplan–Meier survival analysis, the composite event-free survival rates were not statistically significantly different between the three treatment groups (P = 0.26). Comparing the high-efficiency OL-HDF with high-flux dialysis and the low-efficiency OL-HDF, the risk of reaching the primary composite endpoint (death and nonfatal cardiovascular events) was 30% lower for the high-efficiency OL-HDF relative to high-flux HD (HR = 0.70, 95% CI 0.46–1.08, P = 0.26). The patients treated with high-efficiency OL-HDF had better overall and cardiovascular survival compared with both low-efficiency HDF and high-flux HD (P = 0.03 and 0.002, respectively) (Figure 3A and B). In univariate Cox-regression analysis, the relative risks of high-efficiency OL-HDF treatment versus high-flux HD for overall and cardiovascular survival were 0.54 (95% CI 0.33–0.88, P = 0.01) and 0.31 (95% CI 0.14–0.65, P = 0.002), respectively (Table 6). The effect of high-efficiency OL-HDF on survival remained significant in adjusted Cox-regression analysis. The high-efficiency OL-HDF was associated with a 46% risk reduction for overall mortality [RR = 0.54 (95% CI

Table 5. Comparison of the patients treated with high-flux HD, high- and low-efficiency OL-HDF

	High-Flux HD n = 391	Low-efficiency HDF (RF ≤ 17.4 L), n = 196	High-efficiency HDF (RF > 17.4 L) n = 195	P
Replacement fluid (L/session)	–	16.2 ± 1.0	18.1 ± 0.68	–
Baseline parameters				
Age (years)	56.5 ± 14.9	56.9 ± 11.6	55.8 ± 13.8	0.69
Gender (F, %)	42	44	38	0.55
Time on HD (months)	58.7 ± 46.1	60.9 ± 45.8	53.6 ± 40.8	0.23
Diabetes (%)	33	43	33	0.02
CVD history (%)	25	25	29	0.63
AV fistula (%)	95.4	95.4	97.5	0.24
Blood flow rate (mL/min)	294 ± 44	281 ± 38	304 ± 48	0.001
Systolic blood pressure (mmHg)	127 ± 16	128 ± 17	128 ± 15	0.90
Diastolic blood pressure (mmHg)	78 ± 8	77 ± 8	78 ± 7	0.57
Interdialytic weight gain (% BW)	3.47 ± 1.88	3.70 ± 1.57	3.40 ± 1.51	0.17
Urea reduction rate (%)	74.5 ± 6.3	74.3 ± 7.3	75.6 ± 6.1	0.09
eKt/V	1.42 ± 0.25	1.39 ± 0.29	1.47 ± 0.26	0.09
Albumin (g/dL)	3.85 ± 0.38	3.75 ± 0.34	3.90 ± 0.33	<0.001
Haemoglobin (g/dL)	11.4 ± 1.44	11.7 ± 1.6	11.2 ± 1.41	0.002
Phosphate (mg/dL)	4.88 ± 1.48	5.13 ± 1.55	4.72 ± 1.29	0.01
CRP (mg/dL)	1.71 ± 2.36	1.85 ± 2.47	1.50 ± 2.08	0.30
β-2 Microglobulin (mg/L)	26.1 ± 9.7	27.1 ± 7.9	25.7 ± 7.7	0.47
Follow-up parameters				
Systolic blood pressure (mmHg)	126 ± 13	130 ± 15	129 ± 12	0.002
Diastolic blood pressure (mmHg)	77 ± 6	78 ± 7	78 ± 5	0.16
Interdialytic weight gain (% BW)	3.19 ± 1.52	3.87 ± 2.50	3.29 ± 1.22	<0.001
Urea reduction rate (%)	73.2 ± 5.3	73.9 ± 4.7	76.3 ± 4.3	<0.001
eKt/V	1.33 ± 0.19	1.40 ± 0.20	1.47 ± 0.19	<0.001
Albumin (g/dL)	3.99 ± 0.27	3.91 ± 0.23	3.95 ± 0.24	0.004
Haemoglobin (g/dL)	11.5 ± 1.2	11.7 ± 1.2	11.3 ± 1.0	0.006
Phosphate (mg/dL)	4.72 ± 1.01	4.78 ± 1.04	4.54 ± 0.95	0.03
CRP (mg/dL)	1.47 ± 1.52	1.74 ± 2.07	1.43 ± 1.49	0.13
β-2 Microglobulin (mg/L)	27.2 ± 6.8	27.5 ± 6.3	26.7 ± 6.5	0.53
Bicarbonate (mEq/L)	21.9 ± 1.96	22.2 ± 1.4	22.6 ± 1.9	<0.001

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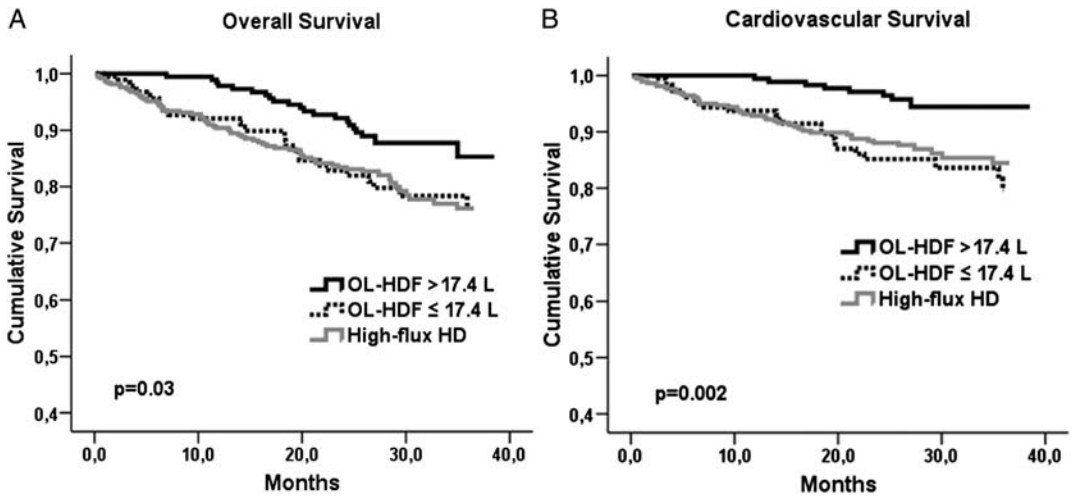

Fig. 3. Overall (**A**) and cardiovascular survival (**B**) among the treatment groups.

Table 6. Unadjusted and adjusted multivariate analysis for predictors of overall and cardiovascular mortality

	Unadjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Overall mortality			
High-flux HD	Reference	Reference	Reference
HDF with RF ≤ 17.4 L	0.99 (0.64–1.53), P = 0.54	1.17 (0.73–1.88), P = 0.36	1.10 (0.68–1.76), P = 0.69
HDF with RF > 17.4 L	0.54 (0.33–0.88), P = 0.01	0.57 (0.33–0.96), P = 0.04	0.54 (0.31–0.93), P = 0.02
Age (per year)		1.05 (1.03–1.07), P < 0.001	1.05 (1.03–1.07), P < 0.001
Presence of diabetes		1.73 (1.15–2.60), P = 0.007	1.88 (1.25–2.84), P = 0.002
Albumin (per g/dL)		–	0.49 (0.28–0.85), P = 0.01
Cardiovascular mortality			
HDF with RF ≤ 17.4 L	1.18 (0.72–1.94), P = 0.50	1.27 (0.75–2.16), P = 0.36	1.28 (0.75–2.19), P = 0.35
HDF with RF > 17.4 L	0.31 (0.14–0.65), P = 0.002	0.29 (0.13–0.65), P = 0.003	0.29 (0.12–0.65), P = 0.003
Age (per year)		1.05 (1.03–1.08), P < 0.001	1.05 (1.03–1.08), P < 0.001
Presence of diabetes		2.03 (1.24–3.34), P = 0.005	2.24 (1.35–3.73), P = 0.002

Model 1: Adjusted for age, gender, diabetes, cardiovascular disease, time on haemodialysis, vascular access, interdialytic weight gain, blood flow rate.
Model 2: Model 1+ haemoglobin, albumin, phosphate and eKt/V.

0.31–0.93) P = 0.02] and 71% risk reduction for cardiovascular mortality [RR = 0.29 (95% CI 0.12–0.65) P = 0.003] compared with high-flux HD.

When the patients treated with OL-HDF were grouped as quartiles of their mean replacement volumes (<16.5 L, 16.5–17.4 L, 17.4–18.0 L and >18 L), the overall survival rates were, respectively, 78.5, 75.5, 82.2 and 90.2% (log-rank: 8.31, P = 0.04), and the cardiovascular survival rates were 81.7, 78.7, 93.6 and 95.4% across the quartiles (log-rank: 15.03, P = 0.002).

Despite comparable BP values at baseline, mean systolic BP level was slightly higher in both OL-HDF groups than in the HD group during the follow-up. The mean IDWG was higher in the low-efficiency OL-HDF group compared with the other groups. The patients in the low-efficiency OL-HDF group had lower albumin but higher haemoglobin levels compared with the other groups. On the other hand, urea reduction rate and eKt/V values were higher; serum phosphate levels were lower in the high-efficiency OL-HDF group than in the other groups. The mean blood flow rates during the follow-up were significantly higher in patients treated with high-efficiency OL-HDF (324 ± 21 mL/min) compared with other groups (301 ± 32 mL/min in low-efficiency HDF and 303 ± 32 mL/min in HD) (P = 0.02).

In diabetic subjects treated with OL-HDF (n = 142), the high-efficiency OL-HDF was associated with better cardiovascular survival compared with the low-efficiency OL-HDF (89.6 versus 66.7%, P = 0.005); primary endpoint and overall survival were similar (P = 0.52 and P = 0.14, respectively). In adjusted analysis including age, gender, time on HD, presence of CVD history and blood flow rate, the high-efficiency OL-HDF was associated with better cardiovascular survival (RR = 0.23, 95% CI 0.07–0.71, P = 0.01).

Discussion

The Turkish OL-HDF study was the first large prospective study to investigate whether OL-HDF is able to reduce death and cardiovascular events better than the currently recommended high-flux HD [18]. The primary outcome measure between the treatment arms was not statistically

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significant. The patient population randomized to OL-HDF showed a relative risk reduction of 18% for the composite primary endpoint of overall mortality and first nonfatal cardiovascular events.

It has to be noted that the statistical power for this analysis was lower than hypothesized during the design of the study and therefore, a type II-error cannot be excluded. In other words, a relevant difference may well exist but was not detectable due to the insufficient statistical power of the study. This is to some extent attributable to the much lower than expected event rate after 36 months; an event-free survival of 74.8% in the standard HD group and 77.6% in the OL-HDF group was observed compared with the anticipated 64%. The enrolled patients were younger than the current European dialysis patient population; therefore, the study population may not be representative for Western Europe. Moreover, the patients had already been on dialysis for some years prior to enrollment, representing a healthier patient population compared with the average dialysis patients. Long dialysis vintage may reflect the lower cardiovascular burden of dialysis patients in our study, being associated with a relatively lower mortality rate (12%), which is possibly also related to younger age, better volume control, high AV fistula use. Additionally, the patients entering the trial had their BP well controlled, with only 13% of them being on antihypertensive, reflecting a good dry weight management, which is known to be an important factor associated with cardiovascular events. The majority (95%) of patients had an AV fistula as their vascular access, and only 28% had elevated phosphate levels, thereby indicating that the study had a lower likelihood of achieving the prespecified risk reduction of 35% by the application of the OL-HDF treatment modality.

Canaud *et al*. [12] reported in their investigation of patients from DOPPS, that a higher substitution volume in OL-HDF was associated with better survival, suggesting the importance of achieved higher convection volumes. OL-HDF with higher substitution volumes is considered high-efficiency OL-HDF by virtue of the fact that larger uraemic toxins are eliminated more effectively. A secondary *post hoc* analysis was therefore carried out by differentiating the patient population of the OL-HDF group into those who received substitution volumes below and above the median of 17.4 L. Patients with higher than this median substitution volume was associated with a trend toward improved outcome in terms of the primary composite endpoint, and showed a significantly better overall and cardiovascular survival compared with high-flux HD. This effect remained significant even after adjusting for confounding factors including blood flow rate. Nevertheless, it cannot be ruled out that the observed effects of the high-efficiency group were attributable to a selection of healthier patients. The results of another randomized controlled trial (CONTRAST study, OL-HDF versus low-flux HD) demonstrated that postdilution OL-HDF with a convection volume over 20 L per session is associated with a 34% of risk reduction for mortality compared with low-flux HD [19], similarly to the current study. It does seem that achieving higher convective volume/higher substitution volume is associated with better survival in postdilution OL-HDF treatment.

It is important to address various factors that determine the ability to achieve high substitution volume. In this study, the target substitution volume of over 15 L has been achieved in 96.7% of cases, with a range between 13.5 and 20 L per session. Theoretically, patient-related and medical staff-related factors might be involved in determining the dose of convective treatment. We found that the blood flow rate and serum albumin were higher, and haemoglobin was lower in patients with higher substitution volumes compared with others. Additionally, the prevalence of diabetes was higher in patients with relatively lower substitution volume. The role of a high blood flow rate to reach higher convection is obvious. Similarly, achieving higher convection volumes was reported to be associated with low hematocrit and/or high serum albumin levels also in the CONTRAST Study, suggesting that high haemoglobin and low albumin levels may attenuate convection by reducing filtration fraction [20]. Another point is that doctors and nurses might not be willing to increase convection volume to avoid annoying high-pressure alarms of machines related to excessive haemoconcentration during treatment.

Previous studies have suggested a lower requirement for erythropoietin in patients treated with OL-HDF [21, 22], although a retrospective study reported no difference between patients treated with predominantly HDF and high-flux HD regarding erythropoietin dose [16]. In our study, despite comparable haemoglobin levels between the groups, the prescribed dose of erythropoietin and the erythropoietin resistance index were significantly lower in the OL-HDF group than in the high-flux HD group. Besides greater elimination of middle-sized molecules that are believed to increase the response to erythropoietin, better microbiological quality of fluids used in OL-HDF procedures may contribute toward reducing the erythropoietin dosage required to maintain haemoglobin levels due to a reduction of systemic inflammation.

Several studies have reported better removal of small-, middle- and large-sized molecules by OL-HDF compared with the other dialysis modalities [7, 8, 15, 23–26]. We also found that small solute clearance was better with the OL-HDF group compared with high-flux HD, as confirmed by higher eKt/V values. Unlike other studies, we were unable to observe a lowering of plasma predialysis β-2 microglobulin levels with OL-HDF compared with high-flux HD [27]. Plasma levels of β-2 microglobulin did not increase in both the groups, and there was no difference between patients treated with high-flux HD and OL-HDF with higher or lower volumes during the follow-up. Compared with low-flux HD, reduced β-2 microglobulin levels were reported in patients treated with OL-HDF [23, 24]. In a prospective and randomized trial, comparing postdilution OL-HDF and high-flux HD for a year study period, a similar pretreatment plasma β-2 microglobulin concentrations were observed in both groups, despite a noticeably higher β-2 microglobulin clearance in the OL-HDF group [8], being in line with our findings. This could be explained by the low distribution volume and slow intercompartmental transfer of β-2 microglobulin [28, 29]. In fact, the surgery rate for carpal tunnel syndrome has been found 42% lower in patients treated with HDF or haemofiltration compared with those treated with HD [30].

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Although a better phosphate clearance has been shown in OL-HDF compared with high-flux HD in small studies [10, 15], others could not confirm this [8]. The mean serum phosphate levels were not different for the patients treated with OL-HDF and high-flux HD in our study. Plasma calcium, calcium-phosphate product, PTH levels and usage of phosphate-binders were also similar between the groups. It has to be noted that phosphate control was already relatively adequate at baseline. The percentage of patients with high phosphate levels was only 28% in our patient population. For this reason, the effect of OL-HDF on serum phosphate level could probably not be observed. However, the mean serum phosphate level was significantly lower in OL-HDF applying higher infusion volume compared with the others, reflecting better phosphate clearance by convective transport.

Some studies reported relatively higher IDWG and/or predialysis BP in OL-HDF [16, 31] and speculated a possibility of positive sodium balance. A recent large prospective study (CONTRAST) did not confirm this [19]. Although higher IDWG and BP were observed in the current study compared with high-flux HD, it should be noted that excellent BP control has been achieved in the OL-HDF group reflected by a mean systolic and diastolic BP 129 ± 13 and 77 ± 6 mmHg, respectively, with a very low frequency of antihypertensive use (11.1%).

The results demonstrate that postdilution OL-HDF is a safe and well-tolerated treatment method in the long term. It is important to note that there was no pyrogenic reaction during the study period, microbiological quality of infusate fluid was always within the target, and the hs-CRP levels were not different between the OL-HDF and the high-flux HD groups.

To conclude, despite the aforementioned constraints of the study, our results nevertheless support the possibility that higher substitution volumes in OL-HDF could provide a survival benefit in HD patients. From this point of view, the study is in line with observational findings of the DOPPS database showing an association with better survival in patients with a higher convection volume [12] and with the results of the *post hoc* analysis recently published CONTRAST study data [19]. The event rates were low compared with our assumptions and remained below the average found in current dialysis populations in Europe. The inclusion of a relatively 'healthy' dialysis population (very low incidence of hypertension, diabetes, hypoalbuminaemia and hyperphosphataemia) might have resulted in less cardiovascular events and, together with the high drop-out rates, could have affected the chance of detecting clinically relevant benefits for the overall OL-HDF patient population. Future studies examining the effects of high-volume OL-HDF on outcomes should consider these considerations.

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Conflict of interest statement. E.O. and A.B. are the members of the scientific advisory board of Fresenius Medical Care, Turkey. G.A., H.T., E.S.O., F.K., M.Y., E.H., M.S.D., C.D., S.D., G.S., M.E.Y. and M.O. declare that they have no conflicts of interest. S.M.A., I.O.I. and M.Z. are employees in Fresenius Medical Care, Turkey.

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Glutathione S-transferase A1, M1, P1 and *T1* null or low-activity genotypes are associated with enhanced oxidative damage among haemodialysis patients

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Abstract

Background. Increased oxidative stress is a hallmark of end-stage renal disease (ESRD). Glutathione *S*-transferases (GST) are involved in the detoxification of xenobiotics and protection of oxidative damage. We hypothesized that genetic polymorphism in antioxidant enzymes *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* is more frequent in ESRD and modulates the degree of oxidative stress in these patients.

Methods. *GSTA1*, *GSTM1*, *GSTP1* and *GSTT1* genotypes were determined in 199 ESRD patients and 199 age- and gender-matched controls. Markers of protein and lipid oxidative damage [thiol groups, carbonyl groups, advanced oxidative protein products, nitrotyrosine, malondialdehyde (MDA) and MDA adducts], together with total oxidant status and pro-oxidant–antioxidant balance were determined.

Results. Individual GST polymorphisms influence vulnerability to both protein and lipid oxidation, with *GSTM1-null* gene variant having the most pronounced effect. Furthermore, a strong combined effect of null/low-activity *GSTM1*, *GSTT1*, *GSTA1* and *GSTP1* genotypes in terms of susceptibility towards oxidative and carbonyl stress was found in ESRD patients. When patients were stratified according to *GSTM1* and *GSTT1*, the highest oxidant damage was noted

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