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Vascular endothelial dysfunction in renal replacement therapy modalities

La disfunción vascular endotelial en las distintas terapias de reemplazo renal

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RESUMEN

Introducción: La disfunción vascular endotelial (DVE) es una causa importante de morbilidad y mortalidad cardiovascular en la fase final de enfermedades renales (ESRD). La endotelina 1 (ET-1) y el óxido nítrico (NO) son sustancias vasoactivas que son afectadas en la ESRD. El objetivo de este estudio es comparar los niveles de suero ET-1 y NO entre receptores de trasplantes de riñón (grupo RTx) y en pacientes que reciben hemodiálisis (grupo HD), hemodiafiltración on line (grupo HDF) así como diálisis peritoneal (grupo PD). **Material y métodos:** Cuarenta y un pacientes, así como veinticinco niños sanos participaron en este estudio. Los niveles del suero ET-1 y NO fueron medidos por ELISA para todos los pacientes en cada control. Los síntomas intradialíticos y el monitoreo ambulatorio de la presión sanguínea fueron evaluados en los grupos HD y HDF. **Resultados:** Cuando los grupos de pacientes fueron comparados de forma separada con el grupo de control, los niveles de ET-1 y NO fueron más elevados en estos grupos de pacientes (p=0.0001). El nivel medio de ET-1 era más bajo en el grupo RTx que en el HDF (p=0.02) mientras que no eran diferentes a aquellos relativos a los grupos PD y HD (343.555ng/l, 593.717ng/l, 546.343ng/l and 589.944ng/l; respectivamente). El grupo RTx tuvo el nivel más bajo en suero de ET-1 y NO en comparación con los grupos PD y HD/HDF, aunque la diferencia no reviste importancia estadística. Los niveles séricos medios de NO no presentaban diferencias entre los grupos HD, HDF, PD y RTx (590.237µmol/l,

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563.084µmol/l, 582.433µmol/l, 438.268µmol/l; respectivamente). Los niveles de ET-1 estaban correlacionados negativamente con los niveles de eGFR, hemoglobina y de calcio sérico, así como correlacionados positivamente con los niveles de PTH. **Conclusión:** Los resultados de este estudio sugieren que la DVE continúa en aquellos pacientes receptores de diferentes terapias de reemplazo renal. Por tanto, concluimos que el trasplante de riñón es una mejor opción comparado con otras modalidades de tratamiento dado que el grupo RTx ha presentado niveles más bajos de ET-1 y NO.

PALABRAS CLAVE: Endotelina-1, óxido nítrico, terapia de reemplazo renal, disfunción vascular endotelial.

ABSTRACT

Introduction: Vascular endothelial dysfunction (VED) is an important cause of cardiovascular morbidity and mortality in end-stage renal disease (ESRD). Endothelin-1 (ET-1) and nitric oxide (NO) are vasoactive substances that are affected in ESRD. **Objectives:** The study aimed to compare serum ET-1 and NO levels in renal transplant recipients (RTx group) and patients receiving hemodialysis (HD group), online-hemodiafiltration (HDF group), and peritoneal dialysis (PD group). **Material and Methods:** Forty-one patients and 25 healthy children were enrolled in the study. Serum ET-1 and NO levels were measured by ELISA in all patients and controls. Intradialytic symptoms and ambulatory blood pressure monitoring were evaluated in HD and HDF groups. **Results**: When each patient group was compared with the control group separately NO and ET-1 levels were higher in patients groups (p=0.0001). Median ET-1 levels were significantly lower in the RTx group than in the HDF group (p=0.02) whereas they were not different than in the PD and HD groups (343.555ng/l, 593.717ng/l, 546.343ng/l, and 589.944ng/l; respectively). RTx group had the lowest level of serum ET-1 and NO comparing the PD and HD/HDF groups although the difference did not reach statistical significance. The median serum NO level was not different between the HD, HDF, PD, and RTx groups (590.237µmol/l, 563.084µmol/l, 582.433µmol/l, 438.268µmol/l; respectively). ET-1 levels were negatively correlated to eGFR, hemoglobin, and serum calcium levels, and positively correlated to PTH levels. **Conclusions:** Our results suggest that VED continues in patients receiving different modalities of renal replacement therapy. We concluded that renal transplantation is superior to other treatment modalities since the RTx group had the lowest levels of ET-1 and NO.

KEYWORDS: Endothelin-1, nitric oxide, renal replacement therapy, vascular endothelial dysfunction

INTRODUCTION

End-stage renal disease (ESRD) is a major disease that requires dialysis or renal transplantation for a patient's survival. The treatment options for patients with ESRD are peritoneal dialysis (PD), hemodialysis (HD), online hemodiafiltration (o-HDF), and renal transplantation. Kidney transplant recipients have the best survival rates among patients undergoing these treatment methods [1]. It has been considered that o-HDF is more effective than HD on solute clearance, hemodynamic stability, and reducing inflammation, cardiovascular mortality, and anemia [2,6].

Additionally, PD is the most common method of dialysis in children, especially in infants. It has been known that PD preserves residual renal function and provides more effective fluid control [7]. Mortality is high in patients with chronic kidney disease (CKD), even in renal transplant recipients. Cardiovascular disease is one of the major causes of death in patients with ESRD and renal transplantation [1]. It has been considered that vascular endothelial dysfunction (VED) is the first sign of the pathogenesis of vascular damage and cardiovascular disease [8]. Endothelium-derived factors, endothelin-1 (ET-1) and nitric oxide (NO), affect the development of VED in CKD and also the progression of CKD to the end-stage [9]. ET-1 is a powerful vasoconstrictor.

Transgenic mice which are overexpressing the ET-1 gene show increase in arterial blood pressure and vascular injury [10]. NO has a vasodilator effect and prevents platelet aggregation as well as inhibits the proliferation of vascular smooth muscle cells. Loss of balance between ET-1 and NO systems has been recognized as an important factor for the

development of VED and cardiovascular risk in CKD [9]. Methods of renal replacement therapy may affect the severity of VED and patient survival in adulthood in various ways.

OBJECTIVES

The study aims to evaluate whether serum ET-1 and NO levels as indicators of VED differ between renal replacement modalities and to determine the relationship between these markers and clinical and laboratory parameters.

MATERIALS AND METHODS

Study population

Eight patients (6 female, 2 male) undergoing maintenance HD were enrolled in the study as hemodialysis group (HD group). The underlying renal diseases were neurogenic bladder (n=3), Bardet Biedl syndrome (n=1), cystinosis (n=2), vesicoureteral reflux (n=1), and unknown (n=1) in the HD group. The mean follow-up duration after the onset of hemodialysis was 35.0±19.7 (3.6-57.7) months. All patients were treated with HD for 3 months after enrollment and then the treatment modality was changed into o-HDF (o-HDF group) for the next 3 months. Intradialytic symptoms were recorded during HD and o-HDF periods and ambulatory blood pressure monitoring was evaluated for 44 hours at the end of HD and o-HDF periods. Intradialytic symptoms including dialysis-associated seizures, muscle cramps, vomiting, dizziness, hypotension episodes, and nausea were recorded for each session of the HD and HDF.

All subjects received HD and o-HDF three times per week for 4 hours. Fresenius Cordiax 5008 machines were used for HD and o-HDF sessions with high-flux membranes. Blood flow rate (Qb) was targeted at least 150 ml/min/m2 and the Qb/dialysate flow rate (Qd) ratio was maintained at 1.2. Convective volume (CV) was targeted at 12-15 L/m2 body surface area and calculated from the sum of replacement volume and ultrafiltration. The same dialysate which contained Na+ 135-140 mmol/l, HCO3- 33 mmol/l, and Ca2+ 1.25 mmol/l was used each HD and o-HDF session. Ultrapure dialysis fluid (< 0.1 colony forming units per ml and < 0.03 endotoxin units per ml) were used for each HD and o-HDF session. Heparin or low-molecular-weight heparin was used as anticoagulation therapy in all patients.

Five patients (4 male, and 1 female) receiving PD were enrolled in the study as a PD group. The underlying renal diseases were posterior urethral valve (n=2), atypical hemolytic uremic syndrome (n=1), C3 glomerulopathy (n=1), and cystic renal disease (n=1) in the PD group. All patients were treated with automated peritoneal dialysis using 1.5% or 1.36% glucose-containing PD solution. The mean follow-up duration after the onset of the peritoneal dialysis was 34.4±14.9 (16.0-54.0) months.

The renal transplantation (Rtx) group consisted of 28 kidney transplant recipients (11 female, 17 male). Five of them had cadaveric, two of them had living-unrelated and the others had living-related kidney donors. The living-related kidney donors consisted of 9 mothers, 10 fathers, 1 grandfather, and 1 grandmother. The underlying renal diseases were congenital anomalies of the kidney and urinary tract (n=12), cystic renal disease (n=4), glomerular kidney disorders (n=8), atypical hemolytic-uremic syndrome (n=2), methyl-malonic acidemia (n=1), familial hypomagnesemia with hypercalciuria, and nephrocalcinosis (n=1). The mean follow-up duration after the renal transplantation was 31.8±24.3 (1-86) months. The mean estimated glomerular filtration rate (eGFR) was 60.9±22.2 (17.9-99.6) ml/1.73m²/min. None of the patients had any clinical event that would affect the graft function in the last three months.

The healthy control group consisted of 25 healthy children (12 female, 13 male, mean age 8.3±4.0 years) with no kidney disease in their history or any acute and chronic diseases at the time of blood sampling.

Ethical statements

This study followed the principles of the Declaration of Helsinki and was approved by the Local University Ethics Committee (2015/366). Informed consent was obtained from the parents of all participants.

Physical examination and laboratory tests

Medical history and clinical findings of patients were recorded, and a physical examination was performed at the time of enrollment. The same auxologist took the height and weight measurements of the patients. Body mass index (BMI) kg/m2 was calculated. Standard deviation

scores (SDS) of BMI were calculated according to national data [11]. Patients with arterial blood pressure over the 95th percentile for age and sex were accepted as having hypertension [12]. Echocardiographic and ocular findings were recorded from the patient file.

Ambulatory blood pressure monitoring was performed for HD and o-HDF groups using Spacelabs 90207 (Spacelabs Healthcare, Hertford, UK). Patients with systolic and or diastolic blood pressure load over 25% were accepted as having ambulatory hypertension [12]. At the end of HD and o-HDF periods, two blood samples were drawn before and after HD/o-HDF sessions in the middle of the week. Blood samples were drawn during the patient's routine outpatient control for the PD and Rtx groups. One sample was taken for PD, Rtx, and healthy control groups. There was no clinical evidence of infection during sampling in patients and the control group.

Hemoglobin, ferritin, urea, creatinine, electrolytes, calcium, phosphate, parathyroid hormone (PTH), total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, ET-1, and NO levels were assessed. The mean estimated GFR (eGFR) was calculated using the Schwartz formula [13].

Blood samples for ET-1 and NO were centrifuged for 10 minutes at 2000xg. Aliquots of serum were stored at – 80 °C for assaying. Serum levels of Endothelin 1 (ET-1) and Nitric Oxide (NO) were assessed by the enzyme-linked immunosorbent assay (ELISA) technique. Serum ET-1 levels were analyzed using Human Endothelin 1 (ET-1) ELISA Kit (Cat no: YHB1082Hu) purchased from YH Biosearch Laboratory following the manufacturer's instructions. Their levels were expressed as ng/L. The intra-assay coefficient of variations (CV) of ET-1 was <7.9%, and the inter-assay CV was <9.1%.

Serum NO levels were analyzed using a Human Nitric oxide (NO) ELISA Kit (Cat no: YHB2160Hu) purchased from YH Biosearch Laboratory following the manufacturer's instructions. Their levels were expressed as μmol/L. The intra-assay and the inter-assay coefficient of variations (CV) of NO were <6.8% and 8.9%, respectively.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows v.22 (IBM Corp., Armonk, NY). Results are expressed as mean ± SD or median (25th and 75th pers) for descriptive data. The normality of the parameters was tested using the Shapiro–Wilk normality test.

Nonparametric tests (Mann-Whitney U test or Kruskal-Wallis test in cases of more than 2 groups) were used for between-groups comparisons. The Chi-square test was used for the comparison of qualitative data. The Wilcoxon test was used to compare the change in NO and ET-1 between HD and HD groups. The relations between variables were analyzed using Spearman's correlation tests. The statistical significance level was established at $p < 0.05$.

RESULTS

Patient groups and control groups were not significantly different in terms of gender ($p > 0.05$). The median age was not different between the HD, o-HDF, and RTx groups ($p > 0.05$) (**Table 1**).

Table 1: Patient's characteristic

	All Patient Group (n=49)	HD Group (n=8)	o-HDF Group (n=8)	PD group (n=5)	Rtx Group (n=28)
Clinical parameters					
Age (years)					
Median	13.4	14.3	14.6	3.6	14.1
Mean±SD (min-max)	12.6±4.5 (2.1-18.5)	13.2±3.3 (8.1-16.8)	13.5±3.3 (8.4-17.1)	6.8±6.2 (2.1-16.8)	13.2±4.2 (3.8-18.5)

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	All Patient Group (n=49)	HD Group (n=8)	o-HDF Group (n=8)	PD group (n=5)	Rtx Group (n=28)
Gender Female/Male	24/25	6/2	6/2	1/4	11/17
SBP mmHg Mean±SD (min-max)	119±18 (80-164)	129±15 (112-149)	119±12 (102-139)	98±13 (80-110)	121±18 (88-164)
DBP mmHg Mean±SD (min-max)	77±16 (45-118)	85±17 (63-118)	79±14 (63-99)	64±11 (50-80)	75±16 (45-111)
BMI (kg/m²) Mean±SD (min-max)	18.9±4.6 (12.8-29.9)	17.9±5.4 (13.6-29.5)	17.9±5.4 (13.6-29.5)	15.1±1.3 (13.0-16.2)	20.2±4.2 (12.8-29.9)
BMI SDS Mean±SD (min-max)	0.0±1.6 (-3.4-4.5)	0.4±1.7 (-1.3-3.3)	0.41±1.67 (-1.28-3.25)	-1.2±1.2 (-3.4--0.3)	0.0±1.6 (-2.9-4.5)
Laboratory parameters					
Hemoglobin g/dl Mean±SD (min-max)	10.9±1.9 (7.3-15.5)	9.2±1.3 (7.3-11.1)	10.0±0.8 (9.1-11.3)	10.6±1.5 (8.4-12.1)	11.6±1.9 (7.6-15.5)
eGFR ml/1.73m²/min Mean±SD (min-max)	41.9.±33.7 (6.7-145.1)	11.9±3.07 (7.8-18.5)	11.4±2.3 (7.8-14.8)	8.4±1.6 (6.7-10.7)	60.8±21.7 (17.8-99.6)
Cholesterol mg/dl Mean±SD (min-max)	174±37 (96-250)	171±43 (96-228)	189±26 (133-213)	199±44 (172-250)	166±35 (96-238)
Triglyceride mg/dl Mean±SD (min-max)	173±120 (56-737)	178±67 (114-319)	165±47 (85-209)	207-124 (94-340)	168±157 (56-737)
HDL mg/dl Mean±SD (min-max)	74±34 (12-159)	58±37 (19-130)	57±24 (26-89)	92±34 (68-130)	86±31 (12-159)
LDL mg/dl Mean±SD (min-max)	70±27 (29-126)	72±30 (41-122)	101±22 (69-126)	66±14 (52-79)	56±19 (29-94)
Bicarbonate mmol/l Mean±SD (min-max)	22.5±3.3 (14.5-34.6)	21.8±2.7 (17.7-25.6)	23.2±5.0 (18.6-34.6)	23.1±2.9 (19.2-25.7)	22.3±2.8 (14.5-28.5)

	All Patient Group (n=49)	HD Group (n=8)	o-HDF Group (n=8)	PD group (n=5)	Rtx Group (n=28)
Ca mg/dl Mean±SD (min-max)	9.6±0.7 (8.0-11.0)	8.8±0.6 (8.0-9.7)	9.3±0.5 (8.6-10.1)	9.8±0.5 (9.1-10.5)	9.9±0.5 (8.7-11.0)
P mg/dl Mean±SD (min-max)	4.5±1.1 (2.1-7.8)	4.2±0.8 (3.1-5.3)	4.7±0.9 (4.0-6.7)	5.8±1.4 (4.3-7.8)	4.3±1.0 (2.1-6.7)
PTH pg/ml Mean±SD (min-max)	293±311 (6-1343)	293.7±159.3 (123-574)	477.4±376.5 (64-1125)	792.8±414.5 (433-1343)	134.6±135.5 (6.0-433.0)
Hypertension Yes/No	30/19	6/2	4/4	2/3	18/10
Anemia Yes/No	28/21	7/1	6/2	3/2	12/16
Hypercholesterolemia Yes/No	5/44	0/8	0/8	1/4	4/24
Hypertriglyceridemia Yes/No	3/46	0/8	0/8	1/4	2/26
Metabolic ascidosis Yes/No	15/34	3/5	4/4	1/4	7/21
Treatment					
Antihypertensive Yes/No	30/19	6/2	4/4	2/3	18/10
Erythropoietin Yes/No	24/26	8/0	8/0	5/0	2/26
Active vitamin D Yes/No	25/24	8/0	8/0	5/0	4/24
Phosphorus chelation Yes/No	25/24	8/0	6/2	5/0	4/24

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	All Patient Group (n=49)	HD Group (n=8)	o-HDF Group (n=8)	PD group (n=5)	Rtx Group (n=28)
CNI Yes/No	28/21	0/8	0/8	0/5	28/0

HD: Hemodialysis, **o-HDF**: Online-hemodiafiltration, **PD**: Peritoneal dialysis, **RTX**: Renal transplantation, **SBP**: Systolic blood pressure, **DBP**: Diastolic blood pressure, **BMI**: Body mass index, **SDS**: Standard deviation score, **SD**: Standard deviation, **eGFR**: Estimated glomerular filtration rate, **HDL**: High density lipoprotein, **LDL**: Low density lipoprotein, **Ca**: Calcium, **P**: Phosphorus, **PTH**: Parathyroid hormone, **CNI**: Calcineurin inhibitors

Only PD patients were younger than the RTx group (p=0.034). The patient's characteristics were given in Table 1. When each patient group was compared with the control group separately, NO and ET-1 levels were higher in the patient groups than in the controls (p=0.0001) (**Tables 2 and 3, Figures 1 and 2**).

Table 2. Serum nitric oxide and endothelin-1 levels of study groups

	All Patient Group	HD Group Before dialysis	HD Group After dialysis	o-HDF Group Before dialysis	o-HDF Group After dialysis	PD Group	RTx Group	Control Group
NO µmol/L Median IQR (25th-75th)	551.9 (224.0-625.2)	590.2 (380.4-611.9)	624.7 (408.6-631-1)	563.1 (459.1-628.2)	627.8 (624.4-631.5)	582.4 (258.2-613.0)	438.3 (108.8-640.2)	56.2 (27.3-125.1)
ET-1 ng/L Median IQR (25th-75th)	550.5 (163.5-605.7)	589.9 (364.5-616.7)	599.8 (428.2-603.9)	593.7 (557.7-625.0)	597.5 (579.3-600.2)	546.3 (300.6-573.8)	343.6 (55.7-591.2)	31.8 (26.7-80.9)

HD: Hemodialysis, **o-HDF**: Online-hemodiafiltration, **PD**: Peritoneal dialysis, **RTX**: Renal transplantation, **NO**: Nitric oxide, **ET-1**: Endothelin-1

Table 3. p values of comparisons between the groups

	NO p	ET-1 p
All patients & Control Group*	**0.0001**	**0.0001**
HD & Control Group*	**0.001**	**0.001**
o-HDF & Control Group*	**0.0001**	**0.0001**
PD & Control Group*	**0.0001**	**0.0001**
RTx & Control Group*	**0.0001**	**0.0001**
HD & o-HDF Group*	0.88	0.51
HD & PD Group*	1.00	0.52
HD & RTx Group*	0.51	0.17
o-HDF & PD Group*	1.00	0.22
o-HDF & RTx Group*	0.28	**0.02**
PD & RTx Group*	0.51	0.39
Before o-HDF & after o-HDF**	0.27	0.14
Before HD & after HD**	0.07	0.47

HD: hemodialysis, o-HDF: online-hemodiafiltration, PD: peritoneal dialysis, RTX: renal transplantation, NO: nitric oxide, ET-1: Endothelin-1

Figure 1: Endhotelin-1 ng/l

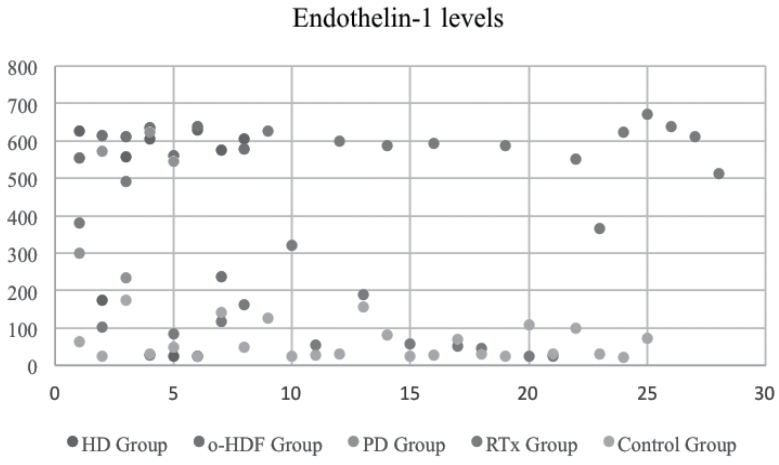

Figure 1: Distribution of serum endothelin-1 levels of hemodialysis (HD) group, online-hemodiafiltration (o-HDF) group, peritoneal dialysis (PD) group, renal transplantation (RTx) group.

Figure 2: Nitric oxide µmol/L

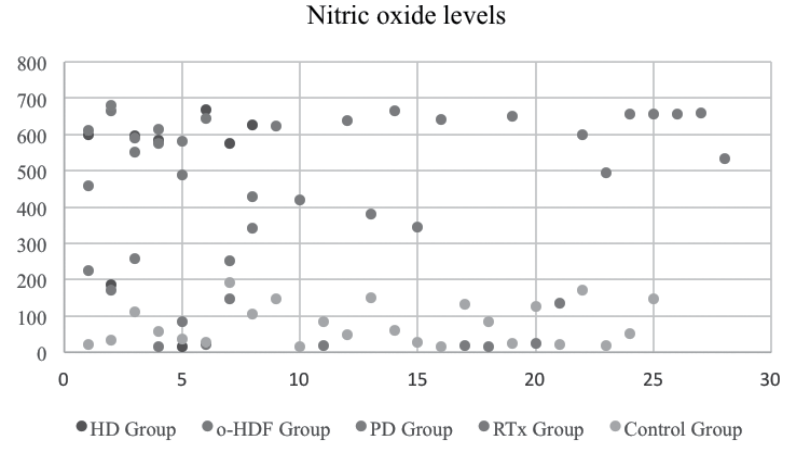

Figure 2: Distribution of serum nitric oxide levels of hemodialysis (HD) group, online-hemodiafiltration (o-HDF) group, peritoneal dialysis (PD) group, renal transplantation (RTx) group.

ET-1 and NO median values of the groups were given in Table 2. The p values of comparisons between the groups were given in **Table 3**.

Comparison of subgroups according to Endothelin-1 levels

Median ET-1 levels were significantly lower in the RTx group than in the o-HDF group (p=0.02) (**Tables 2 and 3**). Also, ET-1 levels were lower in the Rtx group than in the PD and HD groups although this difference was not significant (p>0.05) (**Table 2 and 3, Figure 1**). When we compare HD and o-HDF groups, serum ET-1 levels were not significantly different (p>0.05) (**Tables 2 and 3**). Also, there was no difference between samples obtained before and after HD and o-HDF in terms of ET-1 (p>0.05) (**Tables 2 and 3**). RTx group was separated into two subgroups according to a limit of 100 ng/L of ET-1. [AC1] Among the patients whose ET-1 levels were under this limit, the median age was significantly lower whereas eGFR was significantly higher (p=0.048 and p=0.007; respectively).

Comparison of subgroups according to NO levels

The Rtx group had the lowest level of serum NO although the difference did not reach statistical significance (**Tables 2 and 3, Figure 2**). The median serum NO level was not different between the HD, o-HDF, and PD groups (p>0.05) (**Table 2**). RTx group was separated into two

subgroups according to the limit 100 of µmol/L of NO. [AC2] Median age was significantly lower, yet eGFR was significantly higher in the patients whose NO levels were under the limit (p=0.012 and p=0.042; respectively). Also, there was no difference between samples of before and after HD and o-HDF in terms of median NO levels (p>0.05).

Dialysis vs Rtx

When we compare NO and ET-1 levels of all dialysis patients (HD/o-HDF and PD groups) with the RTx group, the median NO levels were not different (p>0.05). However, median ET-1 levels were significantly lower in the Rtx group (p=0.02) (**Table 4**).

Table 4. Comparison of endothelin-1 and nitric oxide values between dialysis patients and renal transplantation group

	Dialysis patients* n=21	**RTx Group n=28**	**p**
NO µmol/L Median IQR (25th-75th)	582.4 (428.7-613.0)	438.3 (108.8-640.2)	0.22
ET-1 ng/L Median IQR (25th-75th)	576.0 (546.3-615.5)	343.6 (55.7-591.2)	**0.02**

Dialysis patients consist of Hemodialysis, online hemodialysis and peritoneal dialysis group**, RTx**: renal transplantation

HD vs o-HDF

Median ET1 and NO levels were not different between HDF and o-HDF groups (p>0.05). There was no difference between the HD and o-HDF groups in terms of hyperparathyroidism, anemia, metabolic acidosis, hyperlipidemia, inter-dialytic weight gains, and Kt/v (p: 1.00, p: 1.00, p: 1.00, p: 0.47 p: 0.13, p: 0.51, respectively). Also, systolic-diastolic blood pressure and day-night blood pressure measurements were not statistically significant between HD and o-HDF groups (p: 1.00, p: 1.00 respectively). Also, there was no difference between the HD and o-HDF groups in terms of intradialytic symptoms.

Correlations

There was no correlation between age and NO and ET-1 levels (p>0.05). ET-1 levels were negatively correlated to eGFR (r=-0.299, p=0.039), hemoglobin (r=-0.293, p=0.041), and serum calcium levels (r=-0.396, p=0.005), and positively correlated to PTH levels (r=0.327, p=0.037). NO and ET-1 levels were also positively correlated with each other (r=0.876, p<0.0001). NO was negatively correlated to serum calcium levels (r=-0.333, p=0.019). There was no correlation between ET-1, NO levels and phosphorus, triglyceride, cholesterol, systolic and diastolic blood pressure, BMI, and BMI SDS (p>0.05) (**Table 5**).

Table 5. Correlations of between nitric oxide and endothelin-1 levels and clinical laboratory parameters

		Nitric Oxide	**Endothelin-1**
Age (years)	p r	0.218 0.179	0.058 0.273
Hb	p r	0.183 -0.193	**0.041** **-0.293**
Ca	p r	**0.019** **-0.333**	**0.005** **-0.396**
P	p r	0.262 0.163	0.234 0.173
PTH	p r	0.118 0.248	**0.037** **0.327**
eGFR	p r	0.214 -0.183	**0.039** **-0.299**

Table 5 (Continuación)

		Nitric Oxide	Endothelin-1
Triglyceride	p r	0.594 0.092	0.556 0.102
Cholesterol	p r	0.085 0.279	0.170 0.224
Systolic blood pressure	p r	0.551 -0.089	0.694 -0.059
Diastolic blood pressure	p r	0.455 -0.112	0.501 -0.101
BMI	p r	0.198 -0.205	0.163 -0.222
BMI SDS	p r	0.088 -0.270	0.081 -0.275

Hb: Hemoglobin, **PTH**: Parathyroid hormone, **P:** Phosphorus, **Ca:** Calcium, **BMI:** Body mass index, **SDS:** Standard deviation scores

DISCUSSION

The present study demonstrated that ET1 and NO levels as biomarkers of VED were higher in patients who were treated with HD, o-HDF, PD, and RTX. This finding shows that VED exists even in patients on all types of renal replacement therapies and persists even after RTX. When we compare dialyzed patients and the RTX group, the RTX group had lower levels of ET1, suggesting that kidney transplantation is a better treatment option for ESRD in terms of VED. However, the risk of cardiovascular disease and associated death in RTX recipients is still higher than in the general population [14]. ET1 levels have been reported to be higher in adult patients with renal transplantation [15,16]. There are limited data on pediatric RTX recipients. Blazy et al [17] evaluated plasma ET-1 levels in children with RTX and chronic renal failure. They found that ET1 levels in RTX recipients were lower than in hemodialysis children despite being higher than in the healthy control group similar to our results. Murer et al [18]. showed plasma and urine ET1 levels were higher in RTX recipients than in controls, but the difference was not statistically significant. Higher values of ET1 levels in RTX receivers can be attributed to ongoing VED starting with ESRD. There may be additional factors contributing to the VED process in RTX recipients such as ischemia-reperfusion injury, hypertension, immunosuppression therapy, especially cyclosporine [19,22].

Literature data showed conflicting results regarding serum NO levels in adult dialysis patients. Increased, unchanged or decreased levels of NO have been reported in adult patients with different dialysis modalities treated [23,27]. Among the pediatric studies, Ghobrial et al [28] found that NO levels were elevated in children hemodialysis patients in comparison with healthy controls, but the difference did not reach statistical significance. Youssef et al [29] showed that values of NO were higher in hemodialysis children. Some studies demonstrated higher values of NO plasma levels in adult RTX recipients than the healthy controls [30,32]. In our study, the elevation of plasma ET1 levels was accompanied by an elevation of NO in each patient group. Overproduction of ET1 would increase NO and prostacyclin production by ETB receptors [33]. Also, it has been demonstrated that the production of excessive amounts of NO in the uremic milieu is attributed to the overproduction of nitric oxide synthase [34]. Elevated NO might reflect a counter-response to ET1 elevation, uremia, or hypertension in our patients. Moreover, a decrease in clearance of NO may also contribute to the elevation of NO, especially in hemodialysis patients [27].

We demonstrated that ET1 levels were positively correlated to PTH and negatively correlated to hemoglobin, calcium, and eGFR levels. Fujii et al [35] reported that the release of PTH was increased in bovine parathyroid cells with ET1. Chang et al [36] demonstrated that ET1 treatment inhibited PTH mRNA expression in the hyperplastic parathyroid gland of hemodialysis adult patients. Palermo et al [37] found an inverse correlation, although Halaj Zadeh et al [38] showed a positive correlation between ET1 and PTH levels in hemodialysis adult patients. Also, it has been demonstrated that ET1 receptor blockade

did not reduce the PTH levels in uremic rats [39]. Both ET1 and PTH are elevated in CKD patients and our results show an association between them in children with ESRD patients. ET1 appears to have a modulating effect on PTH according to our previous studies and results. It is well known that hyperparathyroidism has a detrimental effect on the cardiovascular system in CKD patients and these effects may be more prominent together with ET1. Additionally, the negative correlation between ET1 and Hb and eGFR levels suggests that vascular endothelial dysfunction is more prominent as CKD progresses.

ET1 has a detrimental effect not only on the cardiovascular system but also on CKD progression [9,33]. In cases affecting renal physiology and the presence of sclerosis in glomeruli, ET1 release from glomerular endothelial cells and podocytes increases [9] ET1 promotes vasoconstriction, glomerular cell injury, and sclerosis via activating ETA receptors [9]. Additionally, ET1 stimulates cell proliferation, and pro-inflammatory cytokines and disrupt podocyte actin-cytoskeleton thus contributing to renal fibrosis [9]. Presence negative correlation between ET-1 and eGFR, and lower eGFR levels in RTX patients with an ET1 level higher than 100 ng/ml may suggest the role of ET1 on the CKD progression. [AC2] It has been demonstrated that blocking of ETA or ETA/ETB receptors attenuated the fibrotic changes in experimental studies. Additionally, some adult clinical studies have reported that ETA receptor blockade has renoprotective effects in CKD [40,42].

Some studies have reported that o-HDF therapy is better than HD in terms of controlling anemia, hyperparathyroidism, inflammation, and intradialytic symptoms [2,6]. It has also been reported that cardiovascular mortality is lower in patients treated with o-HDF than HD [43,44]. Therefore, we evaluated if there was any difference between these two modalities according to serum ET1, NO, intradialytic symptoms, and hypertension, but we could not demonstrate any change in terms of these parameters within a short period of 3 months. Additionally, there was no reduction of ET1 and NO after the HD of o-HDF sessions. There are some studies evaluating the impact of HD sessions at ET1 and NO levels, but the results of these studies in adults are inconsistent with each other [25,44,45]. Warrens et al [45] demonstrated that single HD sessions had no impact on ET1 levels, Ross et al [46] found higher ET1 levels after HD sessions. Tomic et al [25] reported decreases in ET1 levels with HD but NO levels did not change. Among the pediatric studies, Noyan et al [47] reported that HD with acetate-based dialysate and polycarbonate membrane reduced the ET1 levels but HD with bicarbonate-based dialysate and polycarbonate membrane or acetate-based dialysate and polysulfone membrane did not change the ET1 levels. Blazy et al [17] demonstrated that ET1 increased in 6/14 children after the dialysis session. The discordance between these studies may arise from the different study populations, study design, and methods of sample storage. Additionally, some studies have reported that UF rate, intra-dialytic hypertension, or hypotension, post-dialytic hyper or hypotension, and inter-dialytic hypertension affect the post-dialysis ET1 or NO levels [48,49].

In conclusion, despite of small sample size, our study has important results. Unlike other studies, we compared the treatment methods (HD, o-HDF, PD, RTx) that have been applied to ESRD in terms of ET1 and NO in children. Among them, the best modality in terms of ET1 and NO was RTx as the lowest values were obtained in the RTx group. Hyperparathyroidism, anemia, and progression of CKD appear to interact with serum ET1 levels. Effective control of anemia, hyperparathyroidism, and preventive treatment for retarding CKD progression may attenuate VED in children with CKD. Therefore, cardiovascular disease in adulthood may be delayed in this population.

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