Clinical Kidney Journal

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LEADING EUROPEAN NEPHROLOGY

Clinical Kidney Journal, 2018, vol. 11, no. 6, 881–888

doi: 10.1093/ckj/sfy043
Advance Access Publication Date: 25 June 2018
Original Article

ORIGINAL ARTICLE

Peritoneal dialysis fluid biocompatibility impact on human peritoneal membrane permeability

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ABSTRACT

Background. We have compared the effects of conventional lactate-based peritoneal dialysis fluid (CPDF) with respect to bicarbonate/lactate-based fluid on peritoneal ultrafiltration (UF) and peritoneal permeability, and on variations on gene expression in cells isolated from effluents of patients' peritoneal bags.

Methods. This was a non-randomized sequential prospective study including all incident peritoneal dialysis (PD) patients ($n = 40$) recruited in our centre. Peritoneal equilibration tests (PETs) were performed using CPDF or BPDF both containing 2.27% glucose during a 48-h interval in four different sequences. Gene expression variation of selected genes was measured by reverse transcription polymerase chain reaction in mesothelial cells obtained from the total drained fluid during the PET.

Results. In the overall study, the use of BPDF was associated with significantly lower mass transfer area coefficient for urea and creatinine, longer accelerated peritoneal examination test times for urea and creatinine, lower total pore area available for exchange over diffusion distance and lower UF. There were no differences in the gene expression of *aquaporins 1–3*, endothelial and inducible nitric oxide synthase (*NOS3* and *NOS2*), or *interleukin-6*. The *SNAIL and E-CADHERIN* gene expression normalized ratio was evaluated in peritoneal effluents of cells obtained from CPDF and BPDF. We observed that the *SNAIL/E-CADHERIN* mRNA ratio decreased when the dialysis sequence started with BPDF and went on to CPDF, but not when the sequence was the opposite.

Conclusion. This study shows that those patients who started PD treatment with BPDF were characterized by a better biocompatibility profile. BPDF associates with lower peritoneal permeability to small molecules and lower UF.

Keywords: bicarbonate/lactate buffered solution, biocompatibility, epithelial-to-mesenchymal transition, mesothelial cells, peritoneal dialysis, peritoneal transport rate

Received: 11.9.2017; Editorial decision: 2.5.2018

INTRODUCTION

Peritoneal dialysis (PD) is an alternative to haemodialysis for the treatment of end-stage renal disease [1]. In the last decade, PD research has concentrated on trying to improve the quality of the technique and to diminish its complications. Today, it is evident that the physiology of the peritoneal membrane (PM) is more complex than it was previously thought to be, and that it is not just a membrane that answers passively to diffusion and convection. The best model to understand the physiology and functionality of the PM is the three-pore model proposed by Rippe [2]. A good knowledge and better understanding of the physiology of the PM has been reflected in treatment strategies and in the development of new peritoneal dialysis fluids (PDFs) with more biocompatible profiles. In patients who begin automated PD (APD), great interindividual variability is observed in small solute and water transport across the PM. The permeability of the PM is a characteristic that can be considered to be determined by the anatomo–physiological idiosyncrasies of each individual. However, functional changes have also been described in relation to the prescribed solution. Peritoneal transport rate often changes after the initiation of PD [3]. Moreover, inflammation is known to be associated with high peritoneal transport, and peritoneal transport rate also increases with time on PD [4].

Conventional lactate-based peritoneal dialysis fluids (CPDFs) such as Dianeal® are non-physiological in composition; new dialysis fluids including Physioneal® have a more neutral pH, are at least partially buffered with bicarbonate and contain a low glucose degradation product (GDP) concentration. Accordingly, in patients who have long-term exposure to different dialysis solutions to enhance peritoneal survival, the usefulness of the available molecular markers should be monitored. In this context, the characterization of *in vivo* early inflammatory markers and the assessment of how these markers correlate with differences in the expression of key genes and proteins that regulate the ultrafiltration (UF) process are relevant in order to address whether Bicarbonate/lactate-based fluids (BPDFs) result in improved *in vivo* performance. Moreover, long-term exposure to PD solutions causes low-grade chronic inflammation and associates with peritoneal dysfunction [4]. Peritoneal dysfunction is accompanied by an ongoing denudation of mesothelial cells (MCs) and includes a wide spectrum of peritoneal structural changes and, ultimately, fibrosis and UF failure [5]. Epithelial-to-mesenchymal transition (EMT) constitutes an early process following PD unsaturation that may result in fibrosis and neovascularization, and is therefore associated with peritoneal dysfunction [5–7]. Some well-characterized intermediates are known to play a central role in the EMT and fibrosis processes. Transforming growth factor-β is a potent pro-fibrotic factor and inducer of EMT [6]. The *Snail* gene products are known to trigger EMT by inhibiting the expression of E-cadherin [7–9], which contributes to the progressive loss of the epithelial phenotype of MCs and to the acquisition of fibroblast-like characteristics [6].

Some recent studies have focused on the comparative physiology of solute transport of BPDs. At this point, we want to extend our knowledge of the BPDs regarding peritoneal solute transport and UF. To this end, we have investigated the profile of buffer handling and PM transport characteristics during the transition phase from new bicarbonate/lactate (Bi/Lac)-based (Physioneal®) to standard lactate-based (Lac) (Dianeal®) PD solutions and *vice versa*. We also aimed to evaluate alterations in the expression patterns of the aquaporin (*AQP1*, *AQP2* and *AQP3*), inducible and endothelial nitric oxide synthase (*iNOS/NOS2* and *eNOS/NOS3*, respectively) and *interleukin-6* (*IL-6*) genes after the transition from Bi/Lac to Lac dialysis solution and vice versa in cells obtained from patients' dialysis solution effluents. We analysed the correlation between PM functional parameters obtained in the peritoneal equilibration test (PETs) and the expression levels of these genes. We evaluated the usefulness of the ratio between the relative *SNAIL* and *E-cadherin* gene expression levels as an EMT risk marker.

MATERIALS AND METHODS

Patients and study design

A non-randomized sequential prospective study was designed for all incident CKD patients at Stage 5 in our centre who initiated substitutive treatment via PD as the first option technique (Figure 1). Patients initiated basal training in automated and manual PD using Physioneal®. Just before the study began, patients were following during a 2-day dialysis scheme consisting of three fluid exchanges per day. The first two exchanges were done with Physioneal® containing 1.36% glucose and the third (the long night exchange) with icodextrin. In diabetic patients, insulin was administered intraperitoneally with the dose fitted to the dose required to obtain a suitable glucose profile. A total of 40 patients were included (26 men and 14 women) with a mean age of 58.5 $\pm$ 14.3 years. The patients were divided into four groups of 10 patients each. Patients carried out two PETs according to the following scheme: in Group 1, the first PET was done using Physioneal® 40 and glucose 2.27%. Then, each patient was subjected to a second PET performed using Dianeal® PD1 within the following 48 h. In Group 2, the first PET was done with Dianeal® PD1 and a concentration of glucose 2.27%, and then the second PET using Physioneal® 35 within the following 48 h. In Group 3, the first PET was done employing Physioneal® 35 and glucose 2.27%, followed by the second PET within 48 h using Dianeal® PD1 and glucose 2.27%. In Group 4, the first PET was performed using Dianeal® PD1 and glucose 2.27%, followed by the second PET using Physioneal® 40 within 48 h. The characteristics of the PD fluids used are shown in Supplementary data, Table S1. The nominal volume infused in every exchange was of 2000 mL. the drained volume was determined by weighing the bag. Written consent was obtained and the study was approved by the Ethics Committee of Hospital Universitario de Gran Canaria Dr Negrín (Gran Canaria, Las Palmas de Gran Canaria, Spain).

Culture of MC from effluents

PD effluents from PET bags were drained into 50 mL centrifuge tubes and peritoneal cells were concentrated by centrifugation. Cell pellets were suspended in low-glucose Dulbecco's Modified Eagle's medium (5.5 mM of glucose) with antibiotics and without foetal bovine serum (FBS), and counted in a Neubauer chamber. Bags from which recovered cells yielded cultures at densities $\geq 1 \times 10^4$ cells/cm^2 were used [10]. Cells were seeded in 60 mm gelatin-coated culture dishes in DMEM medium with 10% FBS, 100 U/mL of penicillin and 100 µL of streptomycin, and incubated at 37°C in a humidified atmosphere with 5% CO_2. Cells were identified as MCs at confluence by evaluating their morphological features using an inverted light microscope as previously described [10, 11].

Analysis of gene expression

Total RNA was extracted following the Chomczynski method [12]. Reverse transcription of mRNA was carried out according to the manufacturer's instructions. Gene expression levels were compared among subjects by the comparative threshold cycle

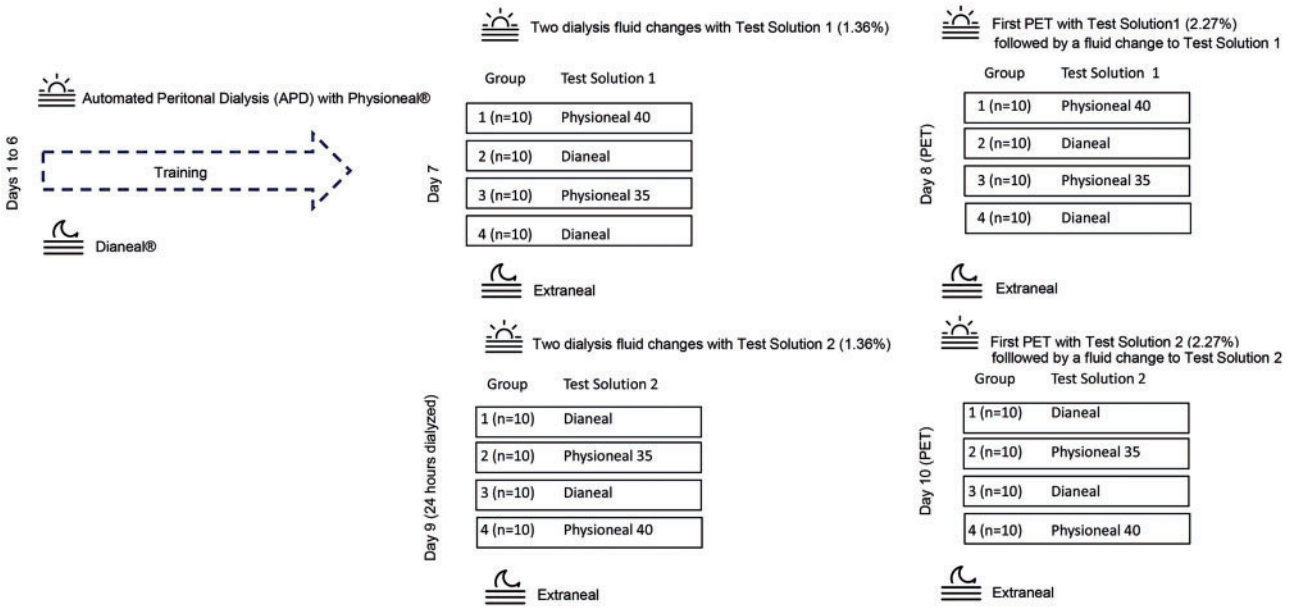

FIGURE 1: Study design.

($\Delta\Delta C_T$) quantitation method. We used a LightCycler and an LC Fast Start DNA Master SYBR Green Kit (Roche Applied Science). *AQP1*, *AQP2*, *AQP3*, *eNOS*, *iNOS* and *IL-6* gene expression were analysed using primer pairs described in the Supplementary data, Table S2. Real-time amplification of the *Snail* and *E-cadherin* gene fragments was performed with primers and conditions previously described [13]. Normalization was performed against a 147-bp amplified fragment of the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene. The normalized *SNAIL/E-CADHERIN* mRNA ratio was used as a marker of epithelial-to-mesenchymal differentiation as described [14]. Depicted data represent the difference between the second and first PETs (PET2–PET1). Polymerase chain reaction product amplification efficiencies were obtained from device software and by plotting C_T differences against pooled cDNA diluted ranging from 1 to 1/100, by confirming that slopes were less than specificities analysed by melting curve analysis and also confirmed by agarose gel electrophoresis.

PM functional evaluation: biochemical analysis

Urea, creatinine and glucose levels were determined in plasma (P) and dialysate samples (D) in modular analytical equipment (Roche Diagnostics). D for urea, glucose (enzymatic method) and creatinine were collected at 0, 20, 60, 120, 140 and 180 min. At the same times, blood samples with anticoagulant were collected, centrifuged and stored at −70°C. Total nitric oxide metabolites were evaluated in P samples filtered through Microcon YM-10 columns (Millipore). Column effluents were evaluated using a colorimetric kit assay according to the manufacturer's instructions (R&D Systems).

PET and mass transfer area coefficient

PET was performed according to the methodology described by Z.J. Twardowski in our dialysis unit to classify patients' permeabilities into one of four established groups: high, average high, average low and low carriers. The peritoneal permeability is classified based on the creatinine curve obtained in the basal balance test and is the value considered for the statistical analysis. Quantitative evaluation of peritoneal permeability was simultaneously determined by means of the mass transfer area coefficient (MTAC) according to the methodology described by Garred *et al.* [15], applying the multiple samples model and the estimation procedure based on the minimization of the sum of squared errors.

UF and absorption of glucose

Total UF was calculated as the difference between the total drained volume measured and the total infused volume (2000 mL) expressed. Glucose absorption was calculated as the difference between the total amounts of instilled glucose according to the removed glucose in the drained fluid and expressed in grams.

Calculation of the sieving effect of sodium (movement of sodium and water)

To calculate the correction for the diffusion of the sieving effect of sodium applicable during the PETs, we follow the methodology described by Westra *et al.* [16]. Accordingly, the transport of small solutes and water across ultra-small pores was calculated in the study.

Calculation of the hydraulic permeability and of the peritoneal surface

The model and methodology used for the calculation of the hydraulic permeability of the PM was the PET data, and is included to the desktop computer calculation software implemented in our unit.

Computer analysis

A self-designed programme was used, compiled with the CLIPPER program (Microsoft), where all the calculations described in the bibliography were integrated so that the results of the studied parameters could be easily obtained. The graphical exit of representation of the curves of the PETs were designed

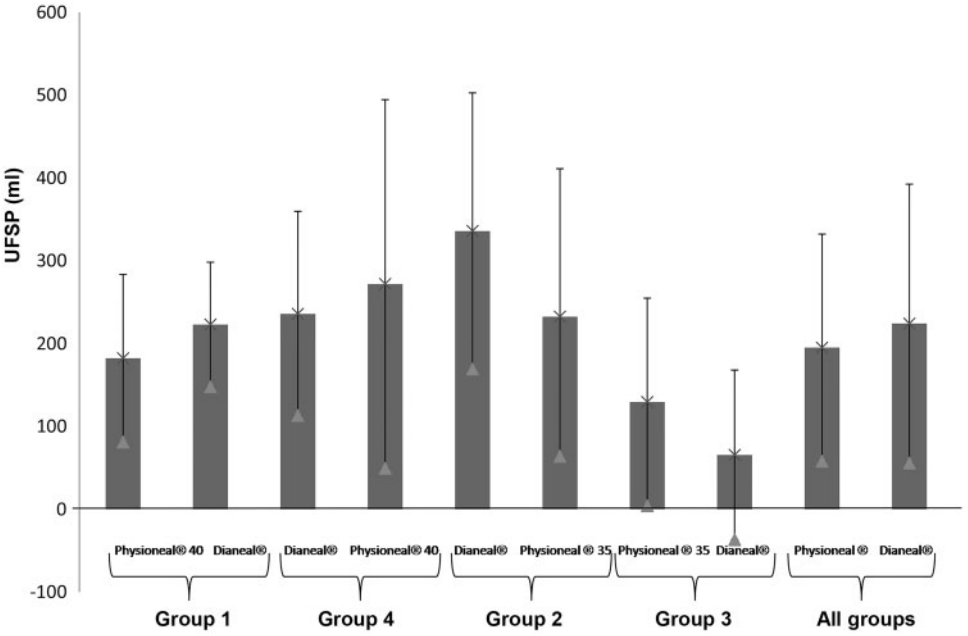

FIGURE 2: Free water transport through ultrasmall pores (UFUSP) in analysed groups according transitions.

Table 1. Main fluid transfer parameters of analysed patients

$n = 40$	Physioneal®	Dianeal®	P
D/P U 240 min	0.88 ± 0.06	0.91 ± 0.06	0.000
D/P Cr 240 min	0.62 ± 0.15	0.65 ± 0.15	0.05
D/P G 240 min	0.41 ± 0.11	0.41 ± 0.11	ns
MTAC U, mL/min	18.51 ± 6.12	21.01 ± 6.17	0.01
MTAC Cr, mL/min	7.41 ± 3.95	8.13 ± 4.50	0.05
MTAC G, mL/min	7.97 ± 3.25	8.18 ± 3.31	ns
TNa (MTAC Cr)	20.78 ± 26.69	27.32 ± 26.71	ns
UF, mL	317 ± 250	393 ± 252	0.05
UFSP, mL	195.17 ± 137.18	224.25 ± 178.39	ns
UFUSP, mL	147.41 ± 81.15	175.43 ± 91.58	0.05
Lpa, mL/min/mmHg	0.06 ± 0.04	0.08 ± 0.04	0.01
$A_0/^{\Delta}x$ average, cm^2	22 862.0 ± 8803.3	25 014.0 ± 8902.2	0.01
APEX time			
Time U, min	109.40 ± 36.36	97.69 ± 37.48	0.01
D/P U	0.62 ± 0.04	0.64 ± 0.04	0.05
Time Cr, min	174.67 ± 60.00	158.42 ± 60.82	0.05
D/P Cr	0.52 ± 0.03	0.53 ± 0.04	0.05

Average dialysate over plasma ratio (D/P); mean hydraulic permeability of the peritoneum by surface area (Lpa); total pore area available for exchange over diffusion distance ($A_0/^{\Delta}x$); urea (U); creatinine (Cr); glucose (G); TNa; ns.

Table 2. Main fluid transfer parameters of Group 1 patients: first PET using Physioneal® 40 and second PET using Dianeal® PD1

$n = 10$	Physioneal® 40	Dianeal®	P
D/P U 240 min	0.87 ± 0.06	0.91 ± 0.07	0.001
D/P Cr 240 min	0.59 ± 0.13	0.66 ± 0.16	0.01
D/P G 240 min	0.42 ± 0.10	0.41 ± 0.11	ns
MTAC U, mL/min	16.07 ± 4.19	20.84 ± 7.03	0.01
MTAC C, mL/min	6.26 ± 3.12	7.99 ± 4.26	0.01
MTAC G, mL/min	7.60 ± 2.44	8.41 ± 3.35	ns
TNa (MTAC Cr)	23.29 ± 18.82	28.70 ± 9.71	ns
UF, mL	355 ± 161	395 ± 134	ns
UFSP	182.37 ± 101.20	223.14 ± 75.20	ns
UFUSP	172.63 ± 67.01	171.86 ± 84.04	ns
Lpa, mL/min/mmHg	0.08 ± 0.02	0.10 ± 0.01	0.05
$A_0/^{\Delta}x$ average, cm^2	20 447.4 ± 6439.2	25 105.9 ± 9383.6	0.01
APEX time curve			
Time U, min	112.82 ± 36.82	102.93 ± 43.09	ns
D/P U	0.63 ± 0.04	0.66 ± 0.05	0.05
Time Cr, min	184.89 ± 50.83	158.82 ± 56.41	0.01
D/P Cr	0.51 ± 0.05	0.55 ± 0.04	0.01

Average dialysate over plasma ratio (D/P); mean hydraulic permeability of the peritoneum by surface area (Lpa); total pore area available for exchange over diffusion distance ($A_0/^{\Delta}x$); urea (U); creatinine (Cr); glucose (G); TNa; ns.

and compiled by the programme QuickBASIC (Microsoft). As the programme uses databases, DBASE III PLUS ('*.DBF') allows these files to be caught by a statistical package capable of recovering '.DBF' files for further analysis.

Statistical analysis

The statistical programmes Sigma of Horus Hardware (Madrid, Spain) and SPSS 12.0 (Chicago, IL, USA) were applied. It was verified that information did not get lost during the procedure and, if it was necessary, files were redefined introducing the precise variables that were not included in the original file in order to adapt the statistical tests according to the design of the project.

The degree of significance P chosen for the univariate analysis, independent of the character of the variable, is the one that corresponds to the level of significance of $P = 0.05$.

As a first step, we checked that the information for the quantitative variables adjusted to normality. After verifying the normality or non-normality of the distribution, the respective parametric or non-parametric statistical tests were applied. As it is a longitudinal and prospective study in which the patients are controls of themselves at two different times, we also used the linear general model of samples (analysis of variance).

Table 3. Main fluid transfer parameters of Group 2 patients: first PET using Dianeal® PD1 and second PET using Physioneal® 35

$n = 10$	Physioneal® 35	Dianeal®	P
D/P U 240′	0.91 ± 0.06	0.93 ± 0.05	ns
D/P Cr 240′	0.65 ± 0.15	0.65 ± 0.16	ns
D/P G 240′	0.41 ± 0.14	0.42 ± 0.09	ns
MTAC U, mL/min	20.78 ± 6.91	23.40 ± 6.50	ns
MTAC Cr, mL/min	8.04 ± 4.30	7.87 ± 4.71	ns
MTAC G, mL/min	8.00 ± 3.77	7.43 ± 3.39	ns
TNa (MTAC Cr)	24.99 ± 32.36	44.18 ± 21.90	0.05
UF, mL	320 ± 298	540 ± 211	0.005
UFSP, mL	232.68 ± 178.61	336.08 ± 166.79	0.05
UFUSP, mL	117.32 ± 79.26	203.92 ± 99.81	0.05
Lpa, mL/min/mmHg	0.06 ± 0.04	0.10 ± 0.03	0.01
$A_0/^{\Delta}x$ average, cm^2	24 743.9 ± 9760.1	25 565.1 ± 9330.2	ns
APEX time curve			
Time U, min	102.88 ± 34.44	93.79 ± 36.82	ns
D/P U	0.64 ± 0.06	0.64 ± 0.03	ns
Time Cr, min	179.28 ± 72.97	165.81 ± 67.50	ns
D/P Cr	0.53 ± 0.03	0.53 ± 0.03	ns

Average dialysate over plasma ratio (D/P); mean hydraulic permeability of the peritoneum by surface area (Lpa); total pore area available for exchange over diffusion distance ($A_0/^{\Delta}x$); urea (U); creatinine (Cr); glucose (G); TNa; ns.

Table 4. Main fluid transfer parameters of Group 3 patients: first PET using Physioneal® 35 and second PET using Dianeal®

$n = 10$	Physioneal® 35	Dianeal®	P
D/P U 240′	0.88 ± 0.07	0.90 ± 0.07	0.08
D/P Cr 240′	0.63 ± 0.19	0.68 ± 0.20	0.01
D/P G 240′	0.38 ± 0.14	0.35 ± 0.13	0.01
MTAC U	19.12 ± 8.08	19.97 ± 5.73	ns
MTAC Cr	8.15 ± 5.16	9.90 ± 5.50	0.05
MTAC G	8.48 ± 4.37	9.70 ± 4.02	0.050086
TNa (MTAC Cr)	8.16 ± 231.03	2.23 ± 21.71	ns
UF, mL	219 ± 285	176 ± 231	ns
UFSP, mL	129.58 ± 125.32	65.68 ± 102.47	ns
UFUSP, mL	139.32 ± 101.21	135.12 ± 97.31	ns
Lpa, mL/min/mmHg	0.04 ± 0.04	0.04 ± 0.03	ns
$A_0/^{\Delta}x$ average, cm^2	24 332.8 ± 17055.7	27 181.5 ± 10559.1	ns
APEX time curve			
Time U, mL	112.59 ± 46.30	87.36 ± 44.46	0.001
D/P U	0.61 ± 0.03	0.61 ± 0.03	ns
Time Cr, mL	159.81 ± 60.49	118.49 ± 54.05	0.001
D/P Cr	0.51 ± 0.03	0.52 ± 0.03	ns

Average dialysate over plasma ratio (D/P); mean hydraulic permeability of the peritoneum by surface area (Lpa); total pore area available for exchange over diffusion distance ($A_0/^{\Delta}x$); urea (U); creatinine (Cr); glucose (G); TNa; ns.

Table 5. Main fluid transfer parameters of Group 4 patients: first PET using Dianeal® PD1 and second PET using Physioneal® 40

$n = 10$	Physioneal® 40	Dianeal®	P
D/P U 240′	0.90 ± 0.05	0.88 ± 0.05	ns
D/P Cr 240′	0.61 ± 0.11	0.62 ± 0.12	ns
D/P G 240′	0.43 ± 0.08	0.41 ± 0.09	ns
MTAC U, mL/min	19.85 ± 5.61	18.07 ± 4.35	ns
MTAC Cr, mL/min	6.74 ± 3.39	7.17 ± 3.19	ns
MTAC G, mL/min	7.20 ± 2.06	7.80 ± 2.44	ns
TNa (MTAC Cr)	34.18 ± 31.27	26.69 ± 25.88	ns
UF, mL	460 ± 279	373 ± 243	ns
UFSP, mL	272.10 ± 222.59	236.03 ± 123.78	ns
UFUSP, mL	190.80 ± 82.84	160.37 ± 74.48	ns
Lpa, mL/min/mmHg	0.07 ± 0.03	0.08 ± 0.04	ns
$A_0/^{\Delta}x$ average, cm^2	21 923.8 ± 6322.0	22 203.4 ± 6516.7	ns
APEX time curve			
Time U, min	102.29 ± 31.00	106.67 ± 30.89	ns
D/P U	0.61 ± 0.02	0.64 ± 0.03	0.05
Time Cr, min	173.49 ± 60.70	186.58 ± 52.39	ns
D/P Cr	0.51 ± 0.04	0.52 ± 0.04	ns

Average dialysate over plasma ratio (D/P); mean hydraulic permeability of the peritoneum by surface area (Lpa); total pore area available for exchange over diffusion distance ($A_0/^{\Delta}x$); urea (U); creatinine (Cr); glucose (G); TNa; ns.

RESULTS

Patients' main characteristics: fluid transfer parameters

The main patient fluid transfer parameters are depicted in Table 1. Patient fluid transfer parameters for the four patient groups are depicted in Tables 2–5. The P-values of the intragroup comparisons are also tabulated. D over P concentration ratios (D/P) for urea, creatinine, glucose and sodium based on a mean of six data points per value, and the estimated MTAC for creatinine, are depicted for the whole population and analysed groups according to transitions. The MTACs were significantly higher for Dianeal® than for Physioneal® (Tables 2–5). These differences occurred during the entire dwell period and were statistically significant for urea, creatinine and sodium. A significant difference between the fluids with higher mean UF values in patients treated with Dianeal® compared with those treated with Physioneal® was observed (Tables 2–5). Group comparisons showed only statistical significance for patients from Group 2, who switched from Dianeal® to Physioneal® 35. In a similar way, UF through small pores was evaluated in each analysed group as well as for all patients. As can be seen in Tables 1–4, the difference was significant for patients who switched from Dianeal® to Physioneal® 35, with the higher mean value in Dianeal® with respect to Physioneal® 35. Free water transport through ultra-small pores (UFUSP) showed a similar pattern (Figure 2). As shown in Figure 2, the significant difference was observed for the patients who switched from Dianeal® to Physioneal® 35 versus all patients. Similarly, mean hydraulic UF coefficients were statistically higher for Dianeal® than those obtained from Physioneal® for all analysed switches except for the Physioneal® 35 to Dianeal® switch (Tables 2–5).

The accelerated peritoneal examination test time curves and accelerated peritoneal examination test time

The accelerated peritoneal examination test (APEX) time is represented by the intersecting point between urea and glucose equilibration curves. The glucose, creatinine and urea equilibration curves were represented in the same graph for each group and solution in order to obtain the time at which these curves cross each other (Figure 3). As can be seen in Figure 3, the patients treated with Dianeal® solutions showed shorter APEX time than those treated with Physioneal®. The difference in the APEX times was smaller when the transition was made from a Physioneal® to Dianeal®, especially when Physioneal® 40 was used, than that observed for Dianeal® to Physioneal® transitions.

Total pore area available for exchange over diffusion distance, $A_0/\triangle x$

Tables 1–3 and 6 show the total pore area available over diffusion distance for each analysed solution and each analysed group. All transitions from Physioneal® to Dianeal® were associated with the

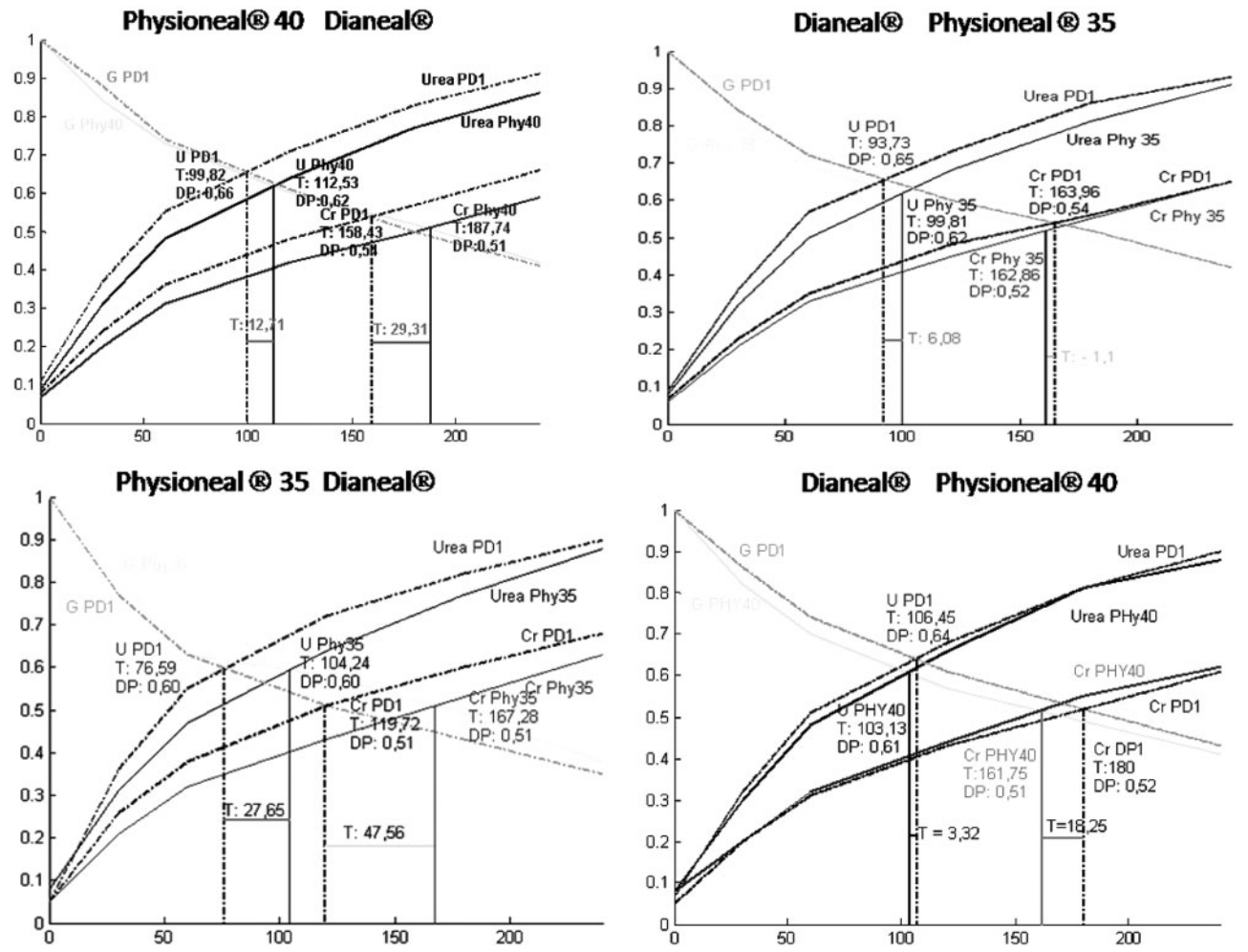

FIGURE 3: Equilibration curves of glucose (G), creatinine (Cr) and urea (U) graphs for studied groups.

Table 6. Main characteristics of studied patients

	Male	Female	Total
Age, years	58.58 ± 14.78	58.43 ± 14.02	58.53 ± 14.33
Diabetes	9	10	19
No diabetes	17	4	21
High	10	2	12
High average	5	2	7
Low average	11	6	17
Low	0	4	4
Total	26	14	40

Data are presented as n or mean ± SD.

increase in $A_0/^{\triangle x}$, whereas the patients that were switched from Dianeal® to Physioneal® did not change their $A_0/^{\triangle x}$.

Gene expression differences

None of the studied transitions was associated with significant changes in the relative expression levels of the analysed genes evaluated in *ex vivo* cultures of cells isolated from bags.

There were no significant differences in the expression levels of the evaluated genes (*AQP1*, *AQP2*, *AQP3*, *NOS2*, *NOS3* and *IL-6*) between the overall Physioneal® and Dianeal® groups.

Assessing for significant relationships

In our study groups, we found a significant negative correlation between expression levels of the *AQP1* and *AQP2* genes and UF through the small pores (UFSP) ($\rho = -0.255$, $P = 0.023$; $\rho = 0.291$, $P = 0.011$ and $\rho = -0.311$, $P = 0.007$, respectively). Moreover, MTAC positively correlated with *AQP2* and *AQP3* gene expression but not with *AQP1* gene expression ($P < 0.05$). Also, specific coefficients for glucose and creatinine MTAC positivity correlated with *AQP2* and *AQP3* expression levels ($P < 0.05$). As has been shown, the water channel AQP2 plays a critical role in tubular sodium and water reabsorption, and in the regulation of extracellular fluid volume both in physiological and pathophysiological conditions. Accordingly, we observed negative correlations between sodium transport coefficients and *AQP2* and *AQP3* gene expression. No significant correlationships were observed among *NOS2*, *NOS3* an *IL-6* gene expression levels with the analysed MTAC and UF parameters.

Significance of the *SNAIL/E-CADHERIN* relative expression levels

The *SNAIL/E-CADHERIN* mRNA ratio was used as a marker of epithelial-to-mesenchymal differentiation. A high ratio is suggestive of a trend towards epithelial-to-mesenchymal

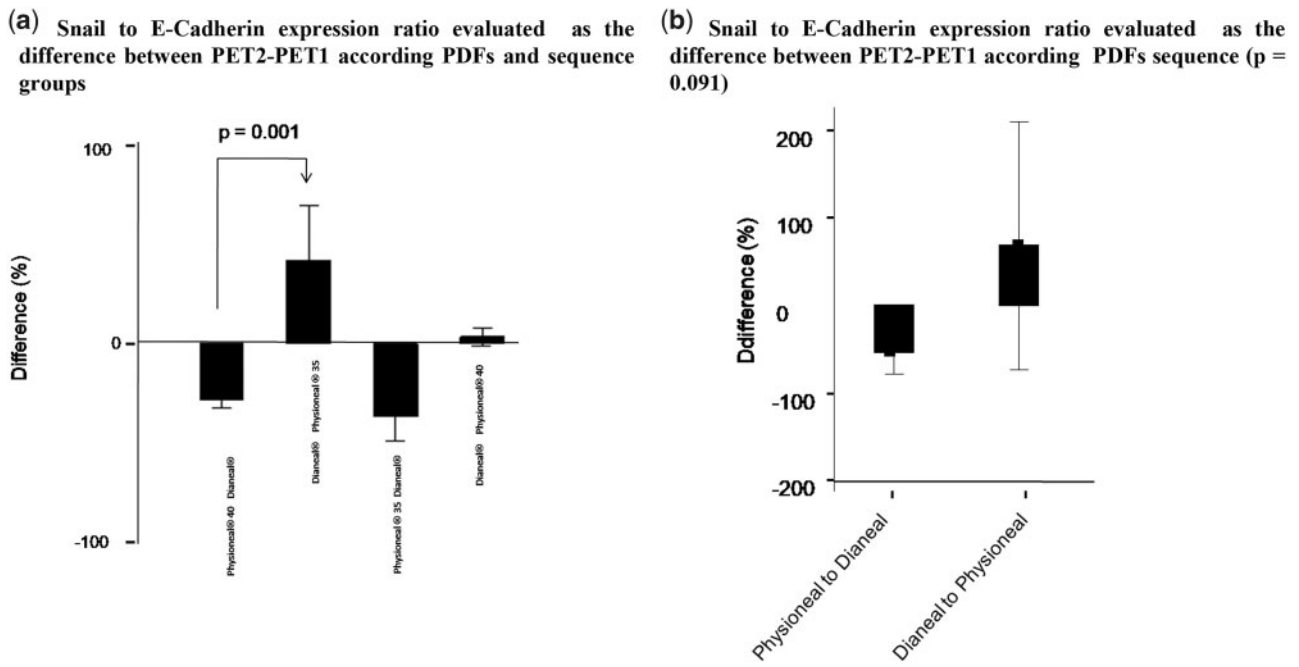

FIGURE 4: *SNAIL* to *E-CADHERIN* expression ratios according sequence groups and PD fluids.

differentiation. Then, the difference between the two sequential PETs was calculated (PET2–PET1). Thus, a positive value for PET2–PET1 indicates higher a trend towards higher epithelial-to-mesenchymal differentiation. When the sequence was Physioneal®-to-Dianeal®, the PET2–PET1 decreased, suggesting a trend towards lower epithelial-to-mesenchymal differentiation on Dianeal® (Figure 4).

DISCUSSION

This work shows that, after performing a PET and regardless of the patients' group assignment, the values of UF and solute transport parameters are higher with Dianeal® than those obtained with Physioneal®. Dianeal® appears to induce functional changes in the permeability of the PM as the values of D/P and MTAC were higher than those obtained with Physioneal®. These changes induced in the PM by Dianeal® persisted and were not modified by Physioneal®, at least not after the short time period that we have evaluated (48 h between test Groups 2 and 4).

Dianeal®-associated hydraulic permeability was also higher than Physioneal®, resulting in a tendency towards greater UF during the PET, whereas no differences were observed in the 24-h UF. Our study supported this by showing that the MTAC means for urea, creatinine and sodium were significantly higher for Dianeal® compared with Physioneal®, and that these differences persisted during the entire dwell period. Moreover, the different exchange capacities of the two PDFs were illustrated by the APEX time. As has been previously suggested, the longer APEX time for Physioneal® than for Dianeal® may have a positive impact on the optimization of the UF capacity. In addition, we applied the three-pore model to compare the two PDFs for dynamic changes in the vascular PM. The total pore area available for exchange over the diffusion distance was significantly larger for Dianeal® than for Physioneal® from the dwell period.

EMT of peritoneal MCs is a pathological process that occurs during PD. The Snail family of transcription factors and Smad-interacting protein 1 regulate E-cadherin. We found that the *SNAIL/E-CADHERIN* mRNA ratio decreased when the sequence was Physioneal® to Dianeal®. This may indicate that Physioneal® may adversely affect the differentiation state of detached MCs *in vivo*. This is not in line with the current understanding of PDF biocompatibility. Ayuzawa *et al.* [17] evaluated peritoneal biopsy specimens from long-term PD patients and concluded that the effect on peritoneal morphology and function of a new biocompatible fluid minimized the progression of peritoneal interstitial sclerosis and hyalinizing vasculopathy. Similarly, Kawanishi *et al.* [18] observed that treatment with PD solutions with low GDPs associates with less PM fibrosis and vascular sclerosis with respect to PD patients treated with conventional solutions with high GDPs. More recently, del Peso *et al.* [19] demonstrated *in vivo* in human biopsies that biocompatible solutions are better tolerated by the peritoneum in the medium- and long-term than conventional solutions. Moreover, the role of *ex vivo* cell function studies and effluent markers in PD patients have also been reviewed [20]. Although the *in vitro* characterization of solution biocompatibility profiles has facilitated the identification of potentially negative factors, these studies have provided little direct information on *in vivo* performance and do not provide definitive evidence of improved patient outcomes when treated with new biocompatible solutions. In any case, our study does have some limitations; we did not assess protein expression and we could not characterize cultured cells as only MCs. Thus, many questions remain to be addressed and the precise interpretation of the *ex vivo* data will require further studies and increased knowledge.

CONCLUSION

This study shows better clinical indices in those patients who started PD treatment with bicarbonate/lactate-based fluid (BPDF). The BPDF was associated with lower peritoneal permeability to small molecules and lower UF. However, *ex vivo* cell gene expression analysis, as well as the evaluation of the *SNAIL/E-CADHERIN* expression ratio as marker of EMT, in PD patients treated with BPDF showed inconclusive results.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

ACKNOWLEDGEMENTS

We want to thank all patients participating in this study. This work was supported in part by research grants from Fundacion Canaria de Investigación Sanitaria (FUNCANIS 6/06) to N. Vega-Diaz and Fundación Mapfre Guanarteme to F. Rodrúguez Esparragón.

CONFLICT OF INTEREST STATEMENT

None declared.

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