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Lipid and Immunophenotypic Profiles in Hemodialysis Patients with Citrate vs. Acetate Dialysates

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Nephrology and Renal Transplantation, Hospital Clínic of Barcelona, 08036 Barcelona, Spain; dmrodriguez@clinic.cat (D.R.-E.); ecuadrado@clinic.cat (E.C.-P.); morantes@clinic.cat (L.M.); mgomezu@recerca.clinic.cat (M.G.); fmaduell@clinic.cat (F.M.)

* Correspondence: jjbroseta@clinic.cat

Abstract: Background: Chronic kidney disease (CKD) is a significant cardiovascular (CV) risk factor, with dialysis-dependent CKD (DD-CKD) patients facing high mortality rates. Hypercholesterolemia is another crucial CV risk factor, typically managed with lipid-lowering therapy, though its efficacy in DD-CKD remains uncertain. Evidence shows mixed results regarding the benefits of statins in these patients. Citrate-based dialysates are known to reduce inflammatory biomarkers compared to acetate-based ones, potentially impacting lipid profiles and immune responses. This study aimed to determine the effects of citrate versus acetate dialysate on lipid profiles and immunophenotypes in DD-CKD patients. **Methods:** This unicentric, cross-over, prospective study included 21 hemodialysis patients (10 males, 11 females, average age 62.25 years). Each patient underwent 24 dialysis sessions (12 with each dialysate) and acted as their own control. Lipid profiles, immunological parameters, and nutritional and inflammatory markers were measured before the last session with each dialysate. **Results:** Compared to acetate dialysate (AD), citrate dialysate (CD) resulted in significantly higher LDL (75.71 vs. 67.05 mg/dL, p=0.042) and HDL (51.19 vs. 47.14 mg/dL, p=0.013), and lower TG (97.86 vs. 110.38 mg/dL, p=0.046). CD also led to higher NK cells (19.24 vs. 16.95%, p=0.035), higher complement C3 levels (115.14 vs. 107.81 mg/dL, p=0.009) and lower CD3+ CD8+ and CD16+56+ lymphocytes. Finally, total lymphocytes were lower with AD than with CD. We found no difference in predialysis nutritional nor inflammatory parameters (IL-6 and hs-CRP) except for ESR, which was higher when subjects used CD than AD. **Conclusion:** There are significant differences in lipid and immunophenotypic profiles that should increase the CV risk of patients who use CD. However, numerous studies have shown no differences, or even a benefit in mortality in certain HD subpopulations that use CD. Further studies are needed to understand if the observed changes may be counterbalanced by other mechanisms potentially provided by citrate (e.g., reducing vascular calcification).

Keywords: lipids; dyslipidemia; acetate; acetate-free; citrate; hemodialysis; dialysate

1. Introduction

Chronic kidney disease (CKD) is a substantial cardiovascular (CV) risk factor, being considered equivalent to having suffered a previous CV event [1–4]. Patients with dialysis-dependent CKD (DD-CKD) have a 5-year mortality rate of approximately 50%, according to some authors [5–7], mainly due to CV causes.

Hypercholesterolemia constitutes another important CV risk factor and is also a therapeutic objective to control in the general population [8–10]. In this sense, low-density lipoprotein (LDL) levels are targeted to a certain threshold according to each patient's calculated risk, given the evidence on lipid control as a preventive measure both for primary and secondary CV events in those with high risk, including CKD patients [11–13]. The association with mortality and cardiovascular disease between other relevant lipid biomarkers and DD-CKD has been studied in triglycerides (TG) [14,15],

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high-density (HDL) [16–18] and very low-density (VLDL) lipoprotein cholesterol [19,20], and lipoprotein (a) [Lp(a)] [21–23], as well as the calculation of the TG/HDL ratio [24].

Unlike other high-cardiovascular-risk populations, the efficacy obtained from lipid-lowering therapy remains uncertain in DD-CKD patients. Two clinical trials and a systematic review failed to prove significant benefits from statins in these patients [25–27]; nonetheless, there is data on the subgroup of dialysis patients from a randomized controlled trial and a real-life retrospective study that show a reduction in major cardiovascular events [28,29]. The discrepancy between findings is probably given to the exclusion in clinical trials of patients with very high LDL levels, their population's heterogeneity, accompanying cardiovascular morbidities, and the higher mortality risk explained in part by chronic inflammation [30–32]. Interestingly, some studies in patients with DD-CKD have reported that high cholesterol is not associated with decreased mortality but with increased survival [33,34]. These findings suggest that LDL blood levels may indicate malnutrition, inflammation, or a sarcopenic state [33].

There is evidence that lymphocyte subpopulations are linked to CV risk. Studies in non-CKD patients indicate that patients with a CD4/CD8 ratio higher than 1.5 [35] and an increased proportion of natural killer (NK) lymphocytes [36] are at a greater risk of CV problems. Also, the complement system, particularly C3 and the C3/C4 ratio, has been associated with CV disease and metabolic disorders [37–39]. It is known that citrate-based dialysates reduce inflammatory biomarkers compared to acetate-based ones, and some authors state that this could be due to reduced oxidative stress and interfere with the immunological inflammation process. Acetate-based dialysates have also been associated with accumulating uremic toxins, membrane biocompatibility, and inflammation [40–42].

The objective of this study is to determine how the use of citrate dialysate, compared to acetate, alters the basic lipid profile and immunophenotype of patients with DD-CKD.

2. Materials and Methods

2.1. Study Design and Population

This is a unicentric, cross-over, prospective study. Patients over 18 years old undergoing post-dilution online hemodiafiltration, who have been on dialysis for at least three months, receiving treatment three times a week for at least four hours each session, and maintaining a stable clinical condition during this period, were eligible for inclusion in the study. Each subject underwent 24 dialysis sessions, 12 with each dialysate acidifier, and acted as their own controls. Blood samples were retrieved predialysis on the last session with each acidifier.

All parameters of the dialysis session (calcium, sodium, and bicarbonate prescriptions; blood and dialysate flows; and dialysis duration), along with medical treatments, were kept constant throughout the study, except for the dialysate acidifier. The details of the dialysate characteristics can be found in Table 1. This study utilized Fresenius 6008 CAREsystem™ dialysis monitors and FX CorDiax™ 60 dialyzers (Fresenius Medical Care, Bad Homburg v.d.H., Germany).

Table 1. Acetate and citrate dialysate components.

Components	Fresenius ACF 3A5	Fresenius Smartbag CA 211.5
Sodium (mmol/L)	140	138
Potassium (mmol/L)	2	2
Calcium (mmol/mL)	1,5	1,5
Magnesium (mmol/mL)	0,5	0,5
Chloride (mmol/mL)	106	109
Acetate (mmol/L)	4	-
Citrate (mmol/L)	-	1
Glucose (g/L)	1	1

 doi:10.20944/preprints202408.1077.v1

2.2. Variables

2.2.1. Lipid Parameters

We registered the lipidic profile by assessing the plasma levels of total cholesterol, LDL, TG, HDL and VLDL lipoprotein cholesterol, and Lp(a), as well as the calculation of the TG/HDL ratio, comparing the use of citrate (CD) vs. acetate (AD) as dialysate acidifier, after twelve sessions with each one.

2.2.2. Immunological Parameters

The studied leucocyte populations were neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio (NLR), natural killers (NK) cells, CD3 positive, CD4 positive, CD8 positive, and CD19 positive count. Complement levels (C3 and C4 levels) and the C3/C4 ratio.

2.2.3. Other Parameters

Blood levels for uric acid, glucose, folic acid, vitamin B12, magnesium, prealbumin, creatin kinase, total proteins, albumin, transferrin, interleukin-6 (IL-6), high-sensitive C-reactive protein (hs-CRP), D-dimer, and erythrocyte sedimentation rate (ESR).

2.3. Statistical Analysis

Quantitative variables are reported with mean and standard deviation when normally distributed or median and 25th and 75th percentiles if skewed. Normal distribution was assessed with the Shapiro-Wilk test, and the comparisons were made with the Student's paired T-test or Wilcoxon's signed-rank test, accordingly. A two-sided p-value ≤ 0.05 was considered statistically significant.

3. Results

Two-hundred and fifty-two sessions were performed with CD and AD in total. Twenty-one participants, consisting of 10 (47.6%) males and 11 (52.4%) females, with an average age of 62.3 ± 13.8 years (ranging from 33.1 to 82.3 years), were included in the study. Dialysis access included arteriovenous fistula for 12 patients and tunneled catheter for 9 patients. Various underlying renal conditions were observed, including diabetic kidney disease (6 patients), autosomal dominant polycystic kidney disease (2 patients), HIV nephropathy (2 patients), hypertensive nephropathy (2 patients), chronic glomerulonephritis (1 patient), chronic pyelonephritis (1 patient), renal cell carcinoma (1 patient), other cystic diseases (1 patient), and undetermined causes (5 patients). Noteworthy comorbidities included cirrhosis with A Child-Pugh score in 1 patient, liver disease with normal function in 5 patients (1 with polycystic hepatorenal disease, 2 with HCV, 1 with HBV, and 1 with HCV-HBV coinfection), and 1 patient who had undergone a liver transplant with normal graft function. Cardiac conditions were present in 3 patients with valve diseases, 2 with chronic ischemic cardiopathy, and 1 with hypertensive cardiomyopathy.

3.1. Lipidic Profile Results

There were significantly lower LDL cholesterol levels with AD compared to CD (AD: 67.05 ± 30.53 v. CD: 75.71 ± 31.01, p= 0.042), higher TG (AD: 110.38 ± 35.79 v. CD: 97.86 ± 29.69, p= 0.046), and higher HDL cholesterol (AD: 51.19 ± 11.25 v. CD: 47.14 ± 11.99, p= 0.013). However, there were no significant differences between predialysis total cholesterol (AD: 140.33 ± 30.56 v. CD: 142.33 ± 33.4, p= 0.624), VLDL (AD: 22.14 ± 7.36 v. CD: 19.67 ± 5.97, p= 0.057), TG/HDL ratio (AD: 2.35 ± 1.16 and CD: 2.29 ± 1.07, p= 0.876), and Lp(a) values (AD: 26.21 ± 30.45 and CD: 27.04 ± 34.21, p= 0.617). See Figure 1 for further details.

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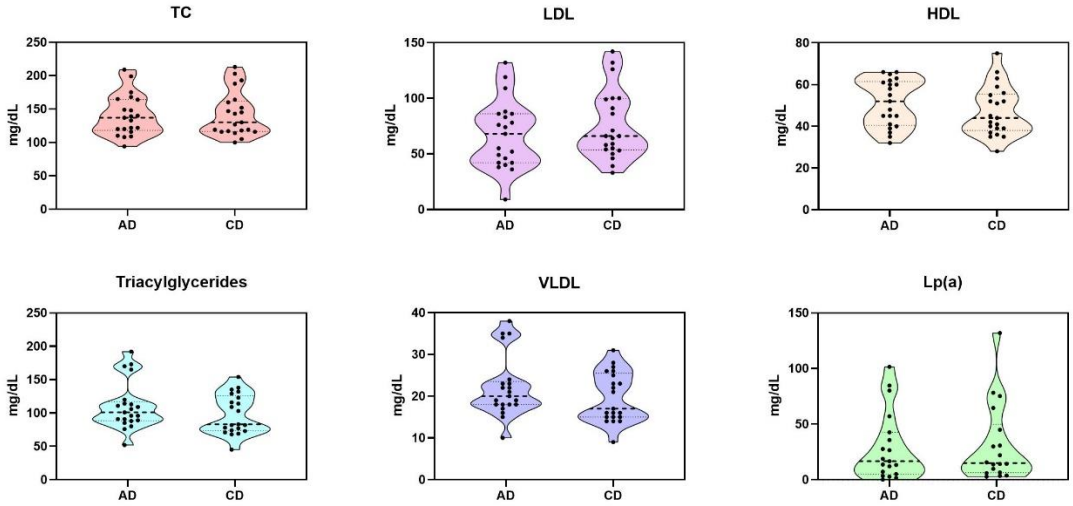

Figure 1. Measured lipid plasma levels after twelve dialysis sessions with each dialysate. AD, acetate dialysate; CD, citrate dialysate; HDL, high density lipoprotein; LDL, low density lipoprotein; lipoprotein (a); TC, total cholesterol; VLDL, very low-density lipoprotein.

3.2. Complement Levels

We found a statistically significant difference in predialysis C3 levels between both dialysates. They were higher when patients were dialyzed with CD than with AD. However, there were no significant differences in C4 levels nor in the C3/C4 ratio (AD: 4.43 ± 0.98 v. CD: 4.5 ± 0.92, p= 0.482). More details in Table 2.

Table 2. Leucocyte and Complement subclasses' concentrations with acetate and citrate dialysates.

Variable	Acetate	Citrate	p-value
Total Leucocytes x 10^3/mm^3, mean ± SD	7.1± 3.9	6.3 ± 2.2	0.03
Neutrophils x 10^3/mm^3, mean ± SD	4.72 ± 3.64	4.09 ± 1.75	0.412
NLR, mean ± SD	3.69 ± 3.01	3.85 ± 1.93	0.776
Lymphocytes x 10^3/mm^3, mean ± SD	1.37 ± 0.46	1.21 ± 0.47	0.037
CD19 %, mean ± SD	121.48 ± 103.26	133.95 ± 88.31	0.423
CD3+ %, mean ± SD	72.9 ± 12.69	68.48 ± 11.86	0.005
CD8+ %, mean ± SD	33.43 ± 14.56	30.67 ± 12.85	0.05
CD4+ %, mean ± SD	517.67 244.83	479.05 193.74	0.272
CD4/CD8, mean ± SD	1.53 ± 1.05	1.57 ± 1.08	0.393
CD56+ CD16+ NK cells %, mean ± SD	16.95 ± 7.85	19.24 ± 8.84	0.035
C3 x 10^3/mm^3, mean ± SD	107.81 ± 19.71	115.14 ± 21.30	0.009
C4 x 10^3/mm^3, mean ± SD	25.48 ± 7.05	26.57 ± 6.78	0.097

CD, cluster of differentiation; NLR, neutrophil to lymphocyte ratio; NK, natural killer; SD, standard deviation.

3.3. Leucocyte Count and Subpopulations

There was a higher number of total leucocytes (AD: 7.1 ± 3.9 v. CD: 6.3 ± 2.2, p= 0.03) and lymphocytes (AD: 1.37 ± 0.46 v. CD: 1.21 ± 0.47, p= 0.037) with AD than with CD. We also found a higher percentage of CD3+ (AD: 72.9 ± 12.69 v. CD: 68.48 ± 11.86, p= 0.0049) and CD8+ T cells (AD: 33.43 ± 14.56 v. CD: 30.67 ± 12.85, p= 0.05), and higher CD56+ CD16+ NK cells (AD: 16.95 ± 7.85 vs CD: 19.24 ± 8.84, p= 0.035). There were no statistically significant differences in mean CD4/CD8 ratios nor the proportion of CD4/CD8 >1.5 with each dialysate (AD: 42.1% v. CD: 57.9%, p= 0.352). There were also no differences observed in predialysis CD19 + B-cells, and CD4+ T-cells, total leucocyte count, neutrophils, NLR, monocytes, nor eosinophils between dialysates. See Table 2 for further information.

 doi:10.20944/preprints202408.1077.v1

3.4. Inflammatory and Nutritional Parameters

There were no statistically significant differences in predialysis IL-6 and hs-CRP between dialysates. However, the ESR was higher when patients used CD than AD (AD: 41.19 ± 22.92 v. CD: 53.62 ± 35.24, p= 0.02). Regarding nutritional parameters, we did not find any significant differences between dialysates in predialysis albumin, prealbumin, total proteins, iron, transferrin, magnesium, iron, folic acid, or vitamin B12 levels. More detailed information available in Table 3.

Table 3. Inflammatory and nutritional parameters with each dialysate.

Variable	Acetate	Citrate	p-value
Glucose (mg/d), mean ± SD	119.15 ± 35.70	126.7 ± 59.52	0.439
Uric acid (mg/d), mean ± SD	5.36 ± 1.43	5.38 ± 1.29	0.943
Amylase (mg/d), mean ± SD	134.05 ± 85.72	125.10 ± 88.86	0.457
CK (mg/d), mean ± SD	82.24 ± 95.51	135.67 ± 204.27	0.129
Total Protein (mg/d), mean ± SD	6.79 ± 0.75	6.77 ± 0.80	0.805
Albumin (mg/d), mean ± SD	4.01 ± 0.40	3.92 ± 0.46	0.119
TSAT (mg/d), mean ± SD	24.90 ± 9.47	29.14 ± 17.19	0.132
Transferrin (mg/d), mean ± SD	177.29 ± 29.93	169.62 ± 32.03	0.066
Magnesium (mg/d), mean ± SD	2.11 ± 0.19	2.05 ± 0.26	0.192
Haptoglobin (mg/d), mean ± SD	154.67 ± 67.57	153.64 ± 75.90	0.912
Vitamin B12 (mg/d), mean ± SD	745 ± 478.39	753.62 ± 435.85	0.865
Folic acid (mg/d), mean ± SD	16.26 ± 8.23	15.90 ± 7.26	0.844
ESR (mg/d), mean ± SD	41.19 ± 22.92	53.62 ± 35.24	0.03
D-dimer (mg/d), mean ± SD	1497.5 ± 1614.27	1490.56 ± 1344.63	0.978
hs-CRP (mg/d), mean ± SD	19.58 ± 59.26	16.46 ± 29.75	0.832
Ferritin (mg/d), mean ± SD	334.67 ± 231.94	398.71 ± 246.55	0.108
Prealbumin (mg/d), mean ± SD	26.21 ± 7.59	24.89 ± 8.089	0.096

CK, creatin kinase; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation; TSAT, transferrin saturation.

4. Discussion

After twelve dialysis sessions with CD, compared to AD, there was a statistically significant decline in TG and HDL and an increase in LDL. Regarding immunology, C3 complement levels were higher, while CD3+ CD8+ and CD16+56+ lymphocytes were lower. Finally, total lymphocytes were lower with AD than with CD. We found no difference in predialysis nutritional nor inflammatory parameters except for ESR, which was higher when subjects used CD than AD.

Few studies have measured the effect of the dialysates' weak acidifier in the lipidic profile, and when done -always as secondary variables- only an increase in LDL been described with the use of CD [43]. Previous studies from the 1980s did not find clinically significant changes in the lipid profile when bicarbonate solutions with reduced concentrations of acetate became commonplace over only acetate solutions [44].

Real-life findings seem to associate lipid values with the opposite of what is expected to be beneficial in the general population. For instance, high TG levels (i.e., > 193 mg/dL) correlate with a lower mortality risk in HD patients [15], while high HDL levels (i.e., > 60mg/dL) appear to correlate with an increased mortality risk in HD patients [18]. In our cohort, the median TG was 101 mg/dL and the highest value registered was 192 mg/dL, not reaching the apparently beneficial value previously published [15], though, it is worth noting that TG levels were significantly higher with AD than with CD. Also, unlike us, a recent study by de Sequera, et al., showed no differences in TG between AD and CD [45]. Regarding HDL, in our cohort we found lower levels with CD than with AD, with 38% of patients having values over 60 mg/dL when using AD while only 14% crossed this threshold while on CD. With regards to LDL, data suggest a U-shaped association with all-cause and cardiovascular mortality risk in peritoneal dialysis patients [46]; however, as previously stated, no clear association has been established with mortality in HD patients.

 doi:10.20944/preprints202408.1077.v1

Further studies must elucidate if the short-term changes induced in the lipidic profile of DD-CKD patients by the dialysate's weak acid translate into clinical implications.

Regarding immunological parameters, there is recent evidence that the use of CD for three months did not affect leucocyte nor total B or T lymphocyte count in comparison to AD in patients on HD [47]. Notably, the same authors measured NLR, which has associated with worse survival in HD patients [48], and, like us, found no differences between dialysates.

Regarding lymphocytic subpopulations, previous studies have shown that there is a U-shaped relationship between the CD4/CD8 T cell- ratio and atherosclerosis progression, being associated with lower ratios in HIV patients (mean ratio of around 0.5) [49–52] and more than 1.5 in Chinese elderly population (mean ratio of around 1.33) [35]. It is reported that early atherosclerotic plaques have ratios below 1, while late atherosclerotic plaques, particularly in late fibroatheroma, are around 1.5 [53]. We found no differences in the proportion of low and high CD4/CD8 ratios between dialysates. However, our population's mean CD4/CD8 ratio was higher than that from the Chinese study despite their subjects being on average older than ours. This higher ratio could potentially translate into a higher CV risk, though more research is needed in dialysis patients.

We found higher total leucocytes and lymphocytes with AD than with CD, predominantly by a higher count of CD3+ and CD8+ lymphocytes, whereas NK cells were higher with CD than with AD. The interpretation of lymphocyte subpopulations needs to be clarified. There is evidence that both CD4 and CD8 decline post-hemodialysis, while their predialysis values remain unchanged compared to healthy populations [54]. This is believed to be in response to poor biocompatibility with the dialyzer membrane or even with the dialysis solution, as postulated by data that has associated acetate with an increased number of activated CD3+CD4+CD69+ T cells than with citrate [47].

LDL receptors play a role in CD8+ T cell activation. Studies in *ldlr* −/− mice and individuals with familial hypercholesterolemia who carry a mutation in LDL receptor show decreased levels of CD8+ cytokine production and cell proliferation [55]. Whether citrate alters LDL receptor function, increasing LDL levels and thus decreasing the number of CD8+ T remains unconfirmed but could partly explain the observed results compared to acetate.

Our cohort found that patients had higher circulating NK cells when exposed to CD than to AD. NK cells constitute another subset of lymphocytes, which may also be necessary in vascular disease [56]. These cells have been isolated from atherosclerotic plaques, particularly expanding the necrotic cores [36]. Regarding circulating levels, a higher percentage of NK cells has been reported in patients with severe atherosclerosis awaiting revascularization [57], elderly patients with coronary disease [58], and has been associated with an increased number of CV and neurological complications after an endarterectomy [59].

Additionally, we found higher TG levels and decreased C3 levels when patients used AD than with CD. These findings may be related, given that data from experimental studies have shown that C3 stimulates glucose uptake and inhibits hormone-sensitive lipase in several cell types [39]. C3 promotes lipophagy, which stimulates VLDL secretion in hepatocytes and balances TG levels in the liver [60]. C3-deficient mice present with glucose intolerance delayed TG clearance and decreased TG storage [39,61,62]. Therefore, C3 has been associated with metabolic disorders and is now recognized as a cardiometabolic risk factor [37]. We also measured the C3/C4 ratio given recent data associating increased serum C3/C4 ratio as a novel marker for recurrent cardiovascular events in acute coronary syndrome [38]. However, we found no differences in this parameter in our cohort.

Multiple sources state lower inflammatory parameters with CD than with AD [40–42,63], but there are some discrepancies in the current literature [47,64]. We found no differences in IL-6 or hs-CRP, though we did find a statistically significant difference in ESR in favor of AD. It is worth noting that most of the previous evidence in this regard comes from patients on HD rather than HDF.

When combining our findings, it appears that there may be an increased CV risk when patients use CD. However, previous studies comparing mortality between CD and AD in HD patients either show no difference or suggest a potential benefit for specific subpopulations [65]. This could be because HD patients behave differently than other subjects, or it could be that CD provides a

 doi:10.20944/preprints202408.1077.v1

counterbalance that mitigates the risk. It has been attributed that CD potentially reduces vascular calcification, which would be a hypothesis to confirm in further studies.

This study has several limitations that should be considered when interpreting the results. Firstly, the single-center design and the small sample size may limit the generalizability of the findings to other populations of DD-CKD patients. Additionally, the relatively short follow-up period of 24 sessions may not be sufficient to observe the long-term effects of the different dialysates on lipid and immunological profiles. Although the crossover design minimizes interindividual variations, the influence of uncontrolled confounding factors, such as changes in diet, or physical activity, during the study, cannot be entirely excluded. Furthermore, the absence of a non-intervention control group makes it challenging to compare the observed changes with the natural progression of these parameters in the absence of dialysate changes. The measurement of inflammatory and nutritional parameters is also subject to biological and technical variability, which could affect the accuracy of the results. Future multicenter studies with larger sample sizes and longer follow-up periods are needed to confirm these findings and better understand the clinical implications of the observed changes.

5. Conclusions

The use of citrate dialysate in DD-CKD patients leads to significant changes in lipid profiles and immune parameters compared to acetate dialysate. Specifically, citrate dialysate increases LDL and HDL levels while reducing TG and alters various lymphocyte subpopulations. These findings suggest that citrate dialysate may have distinct metabolic and immunological effects, potentially influencing cardiovascular risk and inflammation in DD-CKD patients. Additional research is needed to understand the long-term clinical implications of these short-term changes and to optimize dialysis treatment strategies for this high-risk population. To date, the efficacy of lipid-lowering therapy, atherogenic risk factors, and the mechanisms and impacts of dyslipidemia remain unclear in patients with DD-CKD; thus, new methodological approaches like lipidomics are necessary to address this knowledge gap.

Author Contributions: D.R-E., J.J.B., and F.M. participated in the research design. D.R-E. and E.C-P. participated in writing the first version of the manuscript. D.R-E., J.J.B, E.C-P., L.M., and M.G. participated in the performance of the research. D.R-E., J.J.B, and E.C-P. participated in data analysis. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data supporting the findings of this study are available on GitHub (https://github.com/Broseta/Citrate-dialysate.git, accessed on 15 July 2024)

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 doi:10.20944/preprints202408.1077.v1

References

1. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003 Jul;139(2). DOI: 10.7326/0003-4819-139-2-200307150-00013
2. Tonelli M, Muntner P, Lloyd A, Manns BJ, Klarenbach S, Pannu N, et al. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. Lancet. 2012;380(9844):807–14.
3. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation. 2003 Oct;108(17):2154–69.
4. Briasoulis A, Bakri GL. Chronic kidney disease as a coronary artery disease risk equivalent. Curr Cardiol Rep. 2013 Mar;15(3). DOI: 10.1007/S11886-012-0340-4
5. Ahmed M, Alalawi F, Alnour H, Gulzar K, Alhadari A. Five-Year Mortality Analysis in Hemodialysis Patients in a Single-Center in Dubai. Saudi Journal of Kidney Diseases and Transplantation. 2020 Sep;31(5):1062–8.
6. de Arriba G, Avila GG, Guinea MT, Alia IM, Herruzo JA, Ruiz BR, et al. Mortality of hemodialysis patients is associated with their clinical situation at the start of treatment. Nefrología (English Edition). 2021 Jul;41(4):461–6.
7. Saran R, Robinson B, Abbott KC, Agodoa LYC, Bragg-Gresham J, Balkrishnan R, et al. US Renal Data System 2018 Annual Data Report: Epidemiology of Kidney Disease in the United States. American Journal of Kidney Diseases. 2019 Mar;73(3):A7–8.
8. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Jun;139(25):E1082–143.
9. Mach F, Baigent C, Catapano AL, Koskina KC, Casula M, Badimon L, et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Atherosclerosis. 2019 Nov;290:140–205.
10. Alloubani A, Nimer R, Samara R. Relationship between Hyperlipidemia, Cardiovascular Disease and Stroke: A Systematic Review. Curr Cardiol Rev. 2021 Dec;17(6):051121189015.
11. Official Journal of the international Society of nephrology KDIGO Clinical Practice Guideline for Lipid Management in Chronic Kidney Disease KDIGO Clinical Practice Guideline for Lipid Management in Chronic Kidney Disease. 2013 [cited 2024 Jul 3]. Available from: www.publicationethics.org
12. Sarnak MJ, Bloom R, Muntner P, Rahman M, Saland JM, Wilson PWF, et al. KDOQI US commentary on the 2013 KDIGO Clinical Practice Guideline for Lipid Management in CKD. Am J Kidney Dis. 2015 Mar;65(3):354–66.
13. Ferro CJ, Mark PB, Kanbay M, Sarafidis P, Heine GH, Rossignol P, et al. Lipid management in patients with chronic kidney disease. Nat Rev Nephrol. 2018 Dec;14(12):727–49.
14. Soohoo M, Moradi H, Obi Y, Kovesdy CP, Kalantar-Zadeh K, Streja E. Serum triglycerides and mortality risk across stages of chronic kidney disease in 2 million U.S. veterans. J Clin Lipidol. 2019 Sep;13(5):744-753.e15.
15. Huang Y, Zhong Q, Chen J, Qin X, Yang Y, He Y, et al. Relationship of serum total cholesterol and triglyceride with risk of mortality in maintenance hemodialysis patients: a multicenter prospective cohort study. Ren Fail. 2024;46(1). DOI: 10.1080/0886022X.2024.2334912
16. Kopecky C, Ebtehaj S, Genser B, Drechsler C, Krane V, Antlanger M, et al. HDL Cholesterol efflux does not predict cardiovascular risk in hemodialysis patients. Journal of the American Society of Nephrology. 2017 Mar;28(3):769–75.
17. Zewinger S, Speer T, Kleber ME, Scharnagl H, Woitas R, Lepper PM, et al. HDL cholesterol is not associated with lower mortality in patients with kidney dysfunction. J Am Soc Nephrol. 2014 May;25(5):1073–82.
18. Moradi H, Streja E, Kashyap ML, Vaziri ND, Fonarow GC, Kalantar-Zadeh K. Elevated high-density lipoprotein cholesterol and cardiovascular mortality in maintenance hemodialysis patients. Nephrol Dial Transplant. 2014;29(8):1554–62.
19. Yu J, Xia X, Lin T, Huang N, Qiu Y, Yang X, et al. Non-high-density lipoprotein cholesterol and mortality among peritoneal dialysis patients. J Clin Lipidol. 2021 Sep;15(5):732–42.
20. Xie X, Zhang X, Xiang S, Yan X, Huang H, Tian Y, et al. Association of very Low-density Lipoprotein Cholesterol with All-cause and Cardiovascular Mortality in Peritoneal Dialysis. Kidney Blood Press Res. 2017 May;42(1):52–61.
21. Webb AT, Reaveley DA, O'Donnell M, O'Connor B, Seed M, Brown EA. Lipoprotein (a) in patients on maintenance haemodialysis and continuous ambulatory peritoneal dialysis. Nephrology Dialysis Transplantation. 1993 Jan;8(7):609–13.

 doi:10.20944/preprints202408.1077.v1

22. Goldwasser P, Michel MA, Collier J, Mittman N, Fein PA, Gusik SA, et al. Prealbumin and Lipoprotein(a) in Hemodialysis: Relationships With Patient and Vascular Access Survival. American Journal of Kidney Diseases. 1993 Jul;22(1):215–25.
23. Cressman MD, Heyka RJ, Paganini EP, O'Neil J, Skibinski CI, Hoff HF. Lipoprotein(a) is an independent risk factor for cardiovascular disease in hemodialysis patients. Circulation. 1992;86(2):475–82.
24. Gonzáles-Rubianes DZ, Figueroa-Osorio LK, Benites-Zapata VA, Pacheco-Mendoza J, Herrera-Añazco P. Utility of TG/HDL-c ratio as a predictor of mortality and cardiovascular disease in patients with chronic kidney disease undergoing hemodialysis: A systematic review. Hemodial Int. 2022 Apr;26(2):137–46.
25. Wanner C, Krane V, März W, Olschewski M, Mann JFE, Ruf G, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. N Engl J Med. 2005 Jul;353(3):238–48.
26. Fellström BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, et al. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. N Engl J Med. 2009 Apr;360(14):1395–407.
27. Palmer SC, Navaneethan SD, Craig JC, Johnson DW, Perkovic V, Nigwekar SU, et al. HMG CoA reductase inhibitors (statins) for dialysis patients. Cochrane Database Syst Rev. 2013;2013(9). DOI: 10.1002/14651858.CD004289.PUB5
28. Kim JE, Park S, Kim M seok, Kang SJ, Lee JW, Kim KS, et al. Statin initiation and all-cause mortality in incident statin-naïve dialysis patients. Atherosclerosis. 2021 Nov; 337:59–65.
29. Baigent C, Landray MJ, Reith C, Emberson J, Wheeler DC, Tomson C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet. 2011;377(9784):2181–92.
30. Nowak KL, Chonchol M. Does Inflammation Affect Outcomes in Dialysis Patients? Semin Dial. 2018 Jul;31(4):388.
31. Yang Y, Xu Y, Liu S, Lu P, Zhou H, Yang M. The systemic inflammation indexes predict all-cause mortality in peritoneal dialysis patients. Ren Fail. 2023;45(1). DOI: 10.1080/0886022X.2022.2160348
32. Panichi V, Rizza GM, Paoletti S, Bigazzi R, Aloisi M, Barsotti G, et al. Chronic inflammation and mortality in haemodialysis: effect of different renal replacement therapies. Results from the RISCAVID study. Nephrology Dialysis Transplantation. 2008 Jul;23(7):2337–43.
33. Liu Y, Coresh J, Eustace JA, Longenecker JC, Jaar B, Fink NE, et al. Association between cholesterol level and mortality in dialysis patients: role of inflammation and malnutrition. JAMA. 2004 Jan;291(4):451–9.
34. Iseki K, Yamazato M, Tozawa M, Takishita S. Hypocholesterolemia is a significant predictor of death in a cohort of chronic hemodialysis patients. Kidney Int. 2002;61(5):1887–93.
35. Gao P, Rong HH, Lu T, Tang G, Si LY, Lederer JA, et al. The CD4/CD8 ratio is associated with coronary artery disease (CAD) in elderly Chinese patients. Int Immunopharmacol. 2017 Jan;42:39–43.
36. Selathurai A, Deswaerte V, Kanellakis P, Tipping P, Toh BH, Bobik A, et al. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. Cardiovasc Res. 2014;102(1):128–37.
37. Hertle E, Van Greevenbroek MMJ, Stehouwer CDA. Complement C3: an emerging risk factor in cardiometabolic disease. Diabetologia. 2012 Apr;55(4):881.
38. Palikhe A, Sinisalo J, Seppänen M, Haario H, Meri S, Valtonen V, et al. Serum complement C3/C4 ratio, a novel marker for recurrent cardiovascular events. Am J Cardiol. 2007 Apr;99(7):890–5.
39. Phieler J, Garcia-Martin R, Lambris JD, Chavakis T. The role of the complement system in metabolic organs and metabolic diseases. Semin Immunol. 2013 Feb;25(1):47–53.
40. de Sequera Ortiz P, Pérez García R, Molina Nuñez M, et al. Prospective randomised multicentre study to demonstrate the benefits of haemodialysis without acetate (with citrate): ABC-treat Study. Acute effect of citrate. Estudio prospectivo aleatorizado multicéntrico para demostrar los beneficios de la hemodiálisis sin acetato (con citrato): Estudio ABC-treat. Efecto agudo del citrato. Nefrologia (Engl Ed). 2019;39(4):424-433. doi:10.1016/j.nefro.2018.11.002
41. Molina Nuñez M, De Alarcón R, Roca S, Álvarez G, Ros MS, Jimeno C, et al. Citrate versus Acetate-Based Dialysate in On-Line Haemodiafiltration. A Prospective Cross-Over Study. Blood Purif. 2015 May;39(1–3):181–7.
42. Broseta JJ, López-Romero LC, Cerveró A, Devesa-Such R, Soldevila A, Bea-Granell S, et al. Improvements in Inflammation and Calcium Balance of Citrate versus Acetate as Dialysate Buffer in Maintenance Hemodialysis: A Unicentric, Cross-Over, Prospective Study. Blood Purif. 2021 Sep;50(6):914–20.
43. Mahmood D, Stegmayr BG. Haemodialysis with Tinzaparin Versus Dialysate Citrate as Anticoagulation. Blood Purif. 2018 Jul;46(3):257–63.
44. Morin RJ, Srikantaiah M v., Woodley Z, Davidson WD. Effect of hemodialysis with acetate vs bicarbonate on plasma lipid and lipoprotein levels in uremic patients. J Dial. 1980;4(1):9–20.
45. de Sequera P, Pérez-García R, Molina M, et al. Advantages of the use of citrate over acetate as a stabilizer in hemodialysis fluid: A randomized ABC-treat study. Nefrologia (Engl Ed). 2022;42(3):327-337. doi:10.1016/j.nefroe.2021.12.003
46. Wu X, Zhou L, Zhan X, Wen Y, Wang X, Feng X, et al. Low-Density Lipoprotein Cholesterol and Mortality in Peritoneal Dialysis. Front Nutr. 2022 Jul;9. DOI: 10.3389/FNUT.2022.910348/FULL

47. Shen Y, Schmaderer C, Ossadnik A, Hammitzsch A, Carbajo-Lozoya J, Bachmann Q, et al. Immunophenotypic Characterization of Citrate-Containing A Concentrates in Maintenance Hemodialysis: A Pre-Post Study. Int J Nephrol. 2023;2023. DOI: 10.1155/2023/7772677
48. Ahbap E, Sakaci T, Kara E, Sahutoglu T, Koc Y, Basturk T, et al. Neutrophil-to-lymphocyte ratio and platelet-tolymphocyte ratio in evaluation of inflammation in end-stage renal disease. Clin Nephrol. 2016 Apr;85(4):199–208.
49. Castilho JL, Shepherd BE, Koethe J, Turner M, Bebawy S, Logan J, et al. CD4+/CD8+ ratio, age, and risk of serious noncommunicable diseases in HIV-infected adults on antiretroviral therapy. AIDS. 2016 Mar;30(6):899–907.
50. Morell EB, Cabeza JS, Muñoz Á, Marín I, Masiá M, Gutiérrez F, et al. The CD4/CD8 Ratio is Inversely Associated with Carotid Intima-Media Thickness Progression in Human Immunodeficiency Virus-Infected Patients on Antiretroviral Treatment. AIDS Res Hum Retroviruses. 2016 Jul;32(7):648–53.
51. Bernal E, Serrano J, Perez A, Valero S, Garcia E, Marín I, et al. The CD4:CD8 ratio is associated with IMT progression in HIV-infected patients on antiretroviral treatment. J Int AIDS Soc. 2014 Nov;17(4 Suppl 3). DOI: 10.7448/IAS.17.4.19723
52. Lo J, Abbara S, Shturman L, Soni A, Wei J, Rocha-Filho JA, et al. Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. AIDS. 2010 Jan;24(2):243.
53. van Dijk RA, Duinisveld AJF, Schaapherder AF, Mulder-Stapel A, Hamming JF, Kuiper J, et al. A change in inflammatory footprint precedes plaque instability: a systematic evaluation of cellular aspects of the adaptive immune response in human atherosclerosis. J Am Heart Assoc. 2015;4(4). DOI: 10.1161/JAHA.114.001403
54. Lisowska KA, Dębska-Ślizień A, Jasiulewicz A, Heleniak Z, Bryl E, Witkowski JM. Hemodialysis Affects Phenotype and Proliferation of CD4-Positive T Lymphocytes. J Clin Immunol. 2012;32(1):189.
55. Bonacina F, Moregola A, Svecla M, Coe D, Uboldi P, Fraire S, et al. The low-density lipoprotein receptor–mTORC1 axis coordinates CD8+ T cell activation. J Cell Biol. 2022 Nov;221(11). DOI: 10.1083/JCB.202202011
56. Hedrick CC. Lymphocytes in atherosclerosis. Arterioscler Thromb Vasc Biol. 2015 Feb;35(2):253–7.
57. Clerc G, Roux PM. Lymphocyte subsets in severe atherosclerosis before revascularization. Ann Intern Med. 1997;126(12):1004–5.
58. Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK. Decreased natural killer cell activity is associated with atherosclerosis in elderly humans. Exp Gerontol. 2001;37(1):127–36.
59. Kotfis K, Biernawska J, Zegan-Barańska M, Żukowski M. Peripheral Blood Lymphocyte Subsets (CD4+, CD8+ T Cells, NK Cells) in Patients with Cardiovascular and Neurological Complications after Carotid Endarterectomy. Int J Mol Sci. 2015 May;16(5):10077.
60. Li Y, Sha Y, Wang H, He L, Li L, Wen S, et al. Intracellular C3 prevents hepatic steatosis by promoting autophagy and very-low-density lipoprotein secretion. FASEB J. 2021 Dec;35(12). DOI: 10.1096/FJ.202100856R
61. Murray I, Sniderman AD, Havel PJ, Cianflone K. Acylation stimulating protein (ASP) deficiency alters postprandial and adipose tissue metabolism in male mice. J Biol Chem. 1999 Dec;274(51):36219–25.
62. Paglialunga S, Fisette A, Yan Y, Deshaies Y, Brouillette JF, Pekna M, et al. Acylation-stimulating protein deficiency and altered adipose tissue in alternative complement pathway knockout mice. Am J Physiol Endocrinol Metab. 2008 Mar;294(3). DOI: 10.1152/AJPENDO.00590.2007
63. Kossmann RJ, Gonzales A, Callan R, Ahmad S. Increased efficiency of hemodialysis with citrate dialysate: A prospective controlled study. Clinical Journal of the American Society of Nephrology. 2009;4(9):1459–64.
64. Grundström G, Christensson A, Alquist M, Nilsson LG, Segelmark M. Replacement of acetate with citrate in dialysis fluid: a randomized clinical trial of short term safety and fluid biocompatibility. BMC Nephrol. 2013;14(1). DOI: 10.1186/1471-2369-14-216
65. Pérez-García R, Jaldo MT, Puerta M, et al. Hypomagnesaemia in haemodialysis is associated with increased mortality risk: its relationship with dialysis fluid. La hipomagnesemia en hemodiálisis se asocia a mayor riesgo de mortalidad: su relación con el líquido de diálisis. Nefrologia (Engl Ed). 2020;40(5):552-562. doi:10.1016/j.nefro.2020.04.013