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A Pilot Randomized Crossover Trial Assessing the Safety and Short-Term Effects of Pomegranate Supplementation in Hemodialysis Patients

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Abstract

Objective—Oxidative stress and systemic inflammation are highly prevalent in patients undergoing maintenance hemodialysis (MHD) and are linked to excess cardiovascular risk. This study examined whether short-term supplementation with pomegranate juice and extract is safe and well tolerated by MHD patients. The secondary aim was to assess the effect of pomegranate supplementation on oxidative stress, systemic inflammation, monocyte function, and blood pressure.

Design—Prospective, randomized, crossover, pilot clinical trial (NCT01562340).

Setting—The study was conducted from March to October 2012 in outpatient dialysis facilities in the Seattle metropolitan area.

Subjects—Twenty-four patients undergoing MHD (men, 64%; mean age, 61 ± 14 years) were randomly assigned to receive pomegranate juice or extract during a 4-week intervention period. After a washout period, all patients received the alternative treatment during a second 4-week intervention period.

Intervention—Patients assigned to receive pomegranate juice received 100 mL of juice before each dialysis session. Patients assigned to receive pomegranate extract were given 1,050 mg of extract daily.

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Supplementary Data

Supplementary data related to this article can be found at http://dx.doi.org/10.1053/j.jrn.2014.07.006.

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Main Outcome Measures—The main outcome measures were safety and tolerability of pomegranate juice and extract. Additional secondary outcomes assessed included serum lipids, laboratory biomarkers of inflammation (C-reactive protein and interleukin 6) and oxidative stress (plasma F2 isoprostanes and isofurans), monocyte cytokine production, and predialysis blood pressure.

Results—Both pomegranate juice and extract were safe and well tolerated by study participants. Over the study period, neither treatment had a significant effect on lipid profiles, plasma C-reactive protein, interleukin 6, F_2-isoprostane or isofuran concentrations, pre-dialysis systolic or diastolic blood pressure nor changed the levels of monocyte cytokine production.

Conclusions—Both pomegranate juice and extract are safe and well tolerated by patients undergoing MHD but do not influence markers of inflammation or oxidative stress nor affect predialysis blood pressure.

Introduction

Cardiovascular and infectious diseases are the leading causes of death in patients with end-stage renal disease (ESRD).[1] The high cardiovascular morbidity and mortality in patients undergoing maintenance hemodialysis (MHD) cannot be entirely explained by traditional risk factors, and increased oxidative stress has been identified as a key contributor to the pathogenesis of cardiovascular disease in this population.[2] Uremic oxidative stress is biochemically characterized as a state of increased lipid peroxidation, accumulation of unsaturated reactive aldehydes and oxidized thiols, and concomitant depletion of reduced thiol antioxidant groups.[2] Levels of plasma oxidative stress biomarkers are associated with mortality in MHD patients, and accumulating evidence demonstrates that an increase in oxidative stress may play a central role in uremic complications.[3] Chronic systemic inflammation may in turn further exacerbate oxidative stress and along with endothelial dysfunction may act synergistically to accentuate cardiovascular disease and infection-related complications in MHD patients.[4,5]

Given the robust clinical and experimental data linking oxidative stress with excess morbidity and mortality in dialysis patients, there is a compelling rationale for investigating whether novel antioxidant therapies reduce these complications. Polyphenols derived from pomegranate juice have not been adequately studied in clinical trials and represent a potential therapy for hemodialysis patients. Polyphenols have been shown to confer antioxidant protection, reduce platelet aggregation, induce vasorelaxation, and reduce inflammation in humans.[6–8] Several studies suggest that dietary phenols, including those derived from pomegranate juice, may have beneficial effects in patients undergoing dialysis, including reduced infectious and cardiovascular complications, decreased levels of inflammatory biomarkers, improved lipid profiles, and lower systolic blood pressures.[9–11]

The primary objective of this study was to test the hypothesis that 4-week administration of pomegranate juice and/or extract is safe and well tolerated in MHD patients. We also assessed whether 4-week pomegranate juice and/ or extract supplementation influenced biomarkers of oxidative stress or systemic inflammation or affected pre-dialysis blood pressure.

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Methods

Study Design and Participants

This was a prospective, randomized, open-label, crossover trial (NCT01562340). Study participants were recruited from Northwest Kidney Centers outpatient dialysis facilities in the Seattle metropolitan area from March through October 2012 with the following inclusion criteria: ESRD patients receiving thrice-weekly hemodialysis for at least 90 days, aged 18 to 85 years, life expectancy greater than 1 year, and the ability to provide informed consent for study participation. Exclusion criteria included AIDS; active malignancy excluding basal cell carcinoma of the skin; gastrointestinal dysfunction requiring parenteral nutrition; history of poor adherence to hemodialysis or medications; kidney transplant less than 6 months before study enrollment; anticipated live donor kidney transplant over the study duration; patients taking vitamin E supplements (60 IU/day or more), vitamin C (500 mg/day or more), or other antioxidant or nutritional supplements during the 30 days before enrollment; patients hospitalized for more than 5 days within the 30 days preceding enrollment; and patients with a history of a major atherosclerotic event (myocardial infarction, urgent target vessel revascularization, coronary bypass surgery, and stroke). The University of Washington Institutional Review Board approved the study, and all patients provided written informed consent before study enrollment.

Study Procedures

A total of 57 patients were assessed for eligibility, 35 gave consent, and 24 were randomized (Fig. 1). Patients were randomly assigned to 1 of 2 study groups in a 1:1 ratio to either 4 weeks of pomegranate juice (100 mL administered to subjects immediately before each dialysis treatment) followed by 4 weeks of pomegranate juice extract (1,050 mg tablet administered once daily), or 4 weeks of pomegranate juice extract followed by 4 weeks of pomegranate juice (Fig. 2). In each case, the 2 intervention periods were separated by a 4-week washout period. Study outcomes were assessed at baseline, 4, 8, and 12 weeks. Each dose of pomegranate juice and pomegranate juice extract was standardized to deliver 650 gallic acid equivalents, a measure of polyphenol potency.[12] Study products were provided and packaged by POM Wonderful, LLC., Los Angeles, CA. Extract tablets were stored by pharmacy services at Northwest Kidney Centers, Seattle, WA. Study juice was stored in research offices of the Kidney Research Institute, Seattle, WA.

Participants were instructed on proper study medication administration by research coordinators. Accountability for pomegranate extract tablets was assessed at each visit by counting returned dosage units dispensed at the previous visit. Throughout the course of the study, patients received usual dialysis care prescribed by each patient's nephrologist to maintain core quality indicators at or above recommended benchmarks. Adjustments in management of anemia (including iron and erythropoiesis-stimulating agent therapy), blood pressure, and modification to medications were under the direction of each patient's nephrologist.

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Outcomes

The primary outcomes were safety and tolerability of the 2 pomegranate preparations defined as occurrence of adverse events and ability of participants to complete each segment of the trial, respectively. The secondary outcomes were changes in plasma concentrations of C-reactive protein (CRP), interleukin (IL) 6 levels, F_2-isoprostane and isofurans, and changes in the levels of serum cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Additionally, changes in the *in-vitro* production of 3 proinflammatory cytokines by monocytes–IL-1β, IL-6, and tumor necrosis factor α (TNF-α)—and 1 anti-inflammatory cytokine–IL-10—were examined. Predialysis sitting systolic and diastolic blood pressures were recorded before each hemodialysis session as part of routine clinical care. Monocyte cytokine production was assessed in response to 4 different stimuli: Roswell Park Memorial Institute medium (RPMI), lipopolysaccharide (LPS), the synthetic lipopeptide (S)-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser(S)-Lys4-OH, trihydrochloride (Pam3Cys), and flagellin.[13–15]

Analytical Procedures

Blood samples were transported on ice and centrifuged within 1 hour of collection at 4°C at 2,500 RPM for 15 minutes. Aliquots of supernatants were stored at −80°C and thawed before analysis. Total cholesterol was measured by an enzymatic timed end-point method, HDL-C by homogenous colorimetric timed endpoint assay, and triglycerides by enzymatic glycerol phosphate oxidase timed endpoint assay. LDL-C was calculated using the Friedewald equation as total cholesterol minus HDL-C minus triglycerides divided by 5.[16] Plasma CRP levels were determined using an immunoturbidimetric endpoint assay (UniCel DxC 600; Beckman Coulter, Inc, Brea, CA) and IL-6 cytokine concentrations were measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Oxidative stress was quantified by simultaneous measurement of plasma F_2-isoprostane and isofurans by gas chromatography–mass spectrometry analysis.

Human monocytes were isolated from whole blood through initial separation of peripheral blood mononuclear cells (PBMC) using a specialized vacutainer tube containing a gel gradient, followed by centrifugation for 25 minutes at 3,096 RPM at 25°C. The PBMC layer was then removed and cell count performed manually by light microscopy. PBMCs were then mixed with magnetically labeled biotin-conjugated antibodies directed against nonmonocytic cells, and a magnetic separation column was used to deplete magnetically labeled cells. The purified monocytes were counted manually, then mixed and incubated for 20 to 22 hours at 37°C with 4 different stimuli (RPMI, LPS, Pam3Cys, and flagellin). After incubation, samples were centrifuged at 1,500 RPM for 10 minutes at 4°C and cytokine production was detected and quantitated using a bead-based fluorescent multiplexing technique (R&D Systems).

Statistical Analyses

Given the pilot nature of this study, sample size of 24 patients was determined based on feasibility considerations. The demographic characteristics were summarized, stratified by the order of intervention received. Continuous variables are presented as mean and standard

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deviation. All analyses were performed according to intention to treat, with all participants analyzed according to their randomized treatment assignment, irrespective of adherence. The serum CRP and IL-6 levels were below the lower limit of the measurement assay in some subjects. For CRP, the lower limit of the assay was 0.5 mg/L; consequently, values below this level were recoded as 0.25 mg/L. For IL-6, the lower limit of the assay was 3.11 pg/mL; values below this level were recoded as 1.56 pg/mL. The CRP and IL-6 concentrations and the monocyte cytokine production results were transformed on the natural logarithm scale before analysis because these data were not distributed normally.

The assumption that a 4-week washout period was sufficient to eliminate any carryover effect was formally tested by comparing mean values for study outcomes at the start of the first and second treatment periods (Table S1). Additionally, for each outcome, the presence of a period effect was evaluated by comparing the differences of the responses for each sequence group (juice extract or extract juice) with an unpaired 2-sample t-test. No outcomes displayed evidence of a significant carryover or period effect. Thus, regardless of the order of assigned treatments, measurements obtained from patients receiving pomegranate juices were pooled, and measurements obtained from patients receiving pomegranate extract were pooled. Analyses were then carried out for the 2 groups ignoring treatment order. Each patient therefore contributed outcomes to both the juice and extract intervention groups, with the exception of the patients who did not complete the study.

Concentrations of total and HDL-C, triglycerides, LDL-C, CRP, IL-6, F_2-isoprostanes, isofurans, and monocyte cytokine production with and without stimulation before and after each intervention were separately compared using the paired t-test. Differences in laboratory outcome variables at the end of treatment with pomegranate juice was compared with that after treatment with pomegranate extract using linear regression, with inclusion of baseline values for each outcome variable as model covariates. Pre-dialysis blood pressures obtained during the first week of each treatment period were averaged and compared with the average values obtained during the last week of each treatment period using the paired t-test. Given 2 intervention periods and paired data, comparisons were performed using a 2-sided significance level of 0.025.

In the primary analysis, missing continuous variables for patients who did not complete the study were imputed as the mean of the existing values. In sensitivity analyses, to recreate the most extreme possible data sets, outcomes were imputed as best- and worst-case values to simulate the largest and smallest effect estimates compatible with our observed data. In an additional set of sensitivity analyses, missing values were excluded rather than imputed.

All analyses were performed using Stata, version 11 (StataCorp LP, College Station, TX; www.stata.com).

Results

Participant Characteristics

Table 1 shows the baseline demographic data for all study participants stratified by treatment agent during the initial treatment period. Overall, the average age was 61 ± 14

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years; 46% of patients were male, 63% were African American, and 41% had diabetes. Patients who were administered pomegranate extract first were more likely to be white, have diabetes as the etiology of ESRD, and have cardiac disease. Additionally, patients given extract first were less likely to be taking antihypertensive medications or erythropoiesis-stimulating agents (mean weekly dose in patients first administered juice or extract, 4,600 vs. 4,950 units, respectively).

Primary Outcome

Safety and Tolerability—Both study medications were well tolerated by the study participants. When asked, all study participants preferred the pomegranate juice more than the extract. Four patients did not complete participation in the study; the reasons are summarized in Figure 1. There were no study agent-related adverse events or deaths during the course of the study. One patient developed a dental infection between the first and second study visits, which represented the only adverse event during the study (overall adverse event rate, 0.013 events per patient-month).

Secondary Outcomes

Inflammatory Biomarkers, Oxidative Stress, and Lipid Profiles—Table 2 shows mean values for serum lipid, inflammatory and oxidative stress markers before and after treatment, stratified by study medication (pomegranate juice vs. extract). Baseline values for outcome variables were similar in patients treated with pomegranate juice first compared with patients first treated with extract. Neither juice nor extract was associated with a significant change from baseline of any markers of systemic inflammation or oxidative stress.

Table 3 shows the comparison of treatment effect for pomegranate juice and extract. Effect estimates shown are the differences between end of treatment period values comparing pomegranate juice and extract using regression analysis, adjusted for baseline values for each outcome variable. There were no significant differences between the treatment groups with respect to serum lipids or any marker of inflammation. There was a trend toward a larger effect of pomegranate extract on posttreatment concentration of isofurans, with a decrease in isofurans after treatment with pomegranate extract compared with an increase in isofurans after treatment with juice, although this difference was not significant.

Predialysis Blood Pressures—Comparisons of predialysis systolic, diastolic, and mean arterial blood pressures for study participants are shown in Table 4. Mean baseline systolic blood pressure was similar at the beginning of each treatment period (pomegranate juice, 143 mm Hg; extract 140 mm Hg). There was a trend toward small decreases in systolic, diastolic and mean arterial blood pressures after both study treatments, but these changes were not significant.

Monocyte Cytokine Production—Table 5 shows descriptive data for monocyte production of IL-10, IL-1β, IL-6, and TNF-α in response to 4 different adjuvant stimuli (RPMI, LPS, Pam3Cys, and flagellin) before and after each study treatment period, stratified by study medication. As expected, for each cytokine, LPS produced the greatest cytokine

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response, with RPMI as the control medium producing the least intense response. There were no significant differences between pre- and post-treatment cytokine concentrations, irrespective of administered stimulus and treatment agent.

Table 6 compares the treatment effect of pomegranate juice to extract with respect to posttreatment cytokine production. Treatment effect estimates are presented as log unit differences. There were no significant differences between the treatment groups in posttreatment cytokine concentrations.

Sensitivity Analyses

Two sets of sensitivity analyses were performed. In the first set, in comparisons of pre- and post-treatment concentrations of biomarkers and cytokine concentrations, missing data were imputed as the most extreme observed values to create a best-case analysis scenario. Additionally, analyses were repeated excluding missing values. In the second set of analyses, the study participant who developed a concomitant dental infection during the study period was excluded from analysis of the monocyte stimulation cytokine data because of high observed monocyte counts. The results of these analyses were similar to those obtained in the primary analysis with one exception: in the best-case analysis, replacement of missing values with extreme observed values demonstrated a significant reduction in F_2-isoprostane concentration after treatment with both pomegranate juice (P =.01) and extract (P =.009).

Discussion

In this pilot randomized crossover trial of MHD patients, both pomegranate juice and extract were safe and well tolerated. There was no significant difference neither in the effect of pomegranate juice or extract on biomarkers of inflammation or oxidative stress nor on the levels of inflammatory cytokines released from monocytes. Furthermore, neither intervention resulted in a significant change from baseline in concentrations of these biomarkers. We also did not observe a significant difference in the effect of either preparation on serum lipid profiles or on predialysis blood pressures. Nonetheless, our findings highlight the safety and feasibility of pomegranate supplementation as a novel oral antioxidant in patients undergoing MHD and serve as proof of concept for future investigations.

The burden of cardiovascular disease in patients with ESRD is substantial and cannot be entirely explained by the prevalence or severity of traditional disease risk factors such as hypertension and dyslipidemia.[17] Hence, several nontraditional risk factors including systemic inflammation and oxidative stress have been identified as important contributors to this high cardiovascular risk.[2] TNF-α, a strongly proinflammatory cytokine, has been shown to promote endothelial dysfunction and vascular calcification.[18] Similarly, elevated concentrations of IL-6, another proinflammatory cytokine, has been found to be associated with carotid atherosclerosis in hemodialysis patients and predicts poor clinical outcomes in the ESRD population.[19] IL-10, an anti-inflammatory cytokine, has been found to be protective against cardiovascular events in MHD patients.[20] Plasma F_2-isoprostanes, a biomarker of oxidative stress, is 2 to 4 times higher in dialysis patients than in age- and gender-matched healthy subjects.[21] Furthermore, higher levels of markers of lipid

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peroxidation have been linked with impaired endothelium-dependent vasodilation in patients with ESRD.[5] These data along with others suggest a complex and synergistic linkage between systemic inflammation, oxidative stress, and endothelial dysfunction which contributes to cardiovascular disease risk in the ESRD population. Given these findings, there has been interest in exploring the potential for antioxidant therapy to have a favorable impact on the biochemical profile of patients with ESRD with the ultimate goal to improve clinical outcomes and reduce the high burden of cardiovascular and infectious mortality in this population.[22–27]

We chose to study pomegranate products as a source of antioxidants for several reasons. Pomegranate juice is a rich source of potent phenolic antioxidants, which have been demonstrated to have anti-atherogenic, anti-hypertensive, and anti-inflammatory properties. [28] Polyphenol-rich extract from pomegranate fruit has been shown to reduce platelet aggregation and reduce the production of thromboxane A2, a known vasoconstrictor and prothrombotic factor.[29] In nondialysis populations, pomegranate juice consumption has been shown to be associated with improved myocardial perfusion, reduced carotid intima–media thickness, improved blood pressure control, and protects again neonatal cerebral hypoxic-ischemic injury.[30–32] Additionally, concentrated pomegranate juice has been shown to improve lipid profiles in patients with type 2 diabetes.[33]

In patients with ESRD, a pilot clinical trial examined the effects of pomegranate juice administered thrice weekly during hemodialysis treatments. [9,10] In this study, 101 MHD patients at a single center in Israel were randomized to receive 100 mL of pomegranate juice (polyphenol concentration 0.7 mmol per 100 mL) or matching placebo three times weekly with each dialysis session. There was a significant dropout rate of 34% in the treatment group, mostly due to a 15% mortality rate, but also due to loss of patients who disliked the flavor of the juice or who did not believe in the product's efficacy. Nonetheless, the authors noted that treatment with pomegranate juice resulted in reduced levels of proinflammatory cytokines such as IL-6, TNF-α, reduced markers of oxidative stress, improvements in atherosclerotic carotid artery disease, increase in serum albumin, and a reduction in infection-related complications. In a follow-up analysis, the authors also demonstrated reductions in systolic blood pressure and an improvements in triglycerides and HDL-C levels in the group assigned to daily pomegranate juice consumption, effects not seen for patients assigned to placebo.[10] There was no significant difference in the incidence of composite cardiovascular outcomes between the 2 groups.

Two prior placebo-controlled trials have also demonstrated a benefit of antioxidant therapy in reducing cardiovascular events in patients with ESRD.[34,35] In the Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease study, administration of α-tocopherol resulted in significant reduction in the incidence of myocardial infarction and other cardiovascular endpoints compared with placebo.[34] Similarly, a prospective randomized clinical trial of N-acetylcysteine, a thiol-containing antioxidant, in patients undergoing MHD demonstrated a reduction in the incidence of a cardiovascular event composite outcome.[35]

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Although the results of our study do not show a significant effect of treatment on biomarkers of inflammation and oxidative stress, our findings highlight the safety, tolerability, and feasibility of the use of pomegranate supplementation as a novel oral therapy in MHD patients. Given the pilot nature of this trial, the small number of participants in our safety and tolerability study resulted in limited statistical power to demonstrate benefit. Additionally, in this crossover trial, each treatment period lasted only 4 weeks, which may not have been sufficient for the benefits with treatment to manifest; prior clinical trials demonstrating benefit of antioxidant therapy have varied in median follow-up from 12 to 17 months.[9,34,35] Further, patients enrolled in our study had only moderately elevated baseline levels of inflammatory biomarkers (median CRP levels of 7.5 mg/L in patients receiving juice first, and 8 mg/L in patients receiving extract first). It is possible that a benefit with treatment may have been evident in patients with greater severity of systemic inflammation biomarkers at enrollment. We were unable to test multiple dosages of study medications; it is possible that higher doses of polyphenols or more frequent administration of the antioxidant compounds may have led to a greater effect on study outcomes. Additionally, given different physical characteristics of pomegranate juice versus extract tablets, it was not possible to blind patients or staff to which intervention patients received during each study period, and this may have introduced bias. Finally, given the pilot nature of this trial and its short duration, we did not seek to evaluate clinical outcome such as hospitalizations or infections, which may be impacted by treatment even in the absence of change in biomarker levels. Notwithstanding the results of the Secondary Prevention with Antioxidants of Cardiovascular Disease in End-Stage Renal Disease trial and others showing clinical benefit of antioxidant therapies, our study results findings are in line with other recently published investigations such as the Provision of Antioxidant Therapy in Hemodialysis trial, that have failed to show a treatment benefit of antioxidant therapy in MHD patients.[23,27] Despite its limitations, this study is one of the few randomized trials which assess the effect of pomegranate supplementation on laboratory and hemodynamic outcomes in patients with ESRD.

In conclusion, in this 12-week pilot randomized crossover study, we found that pomegranate supplementation was well tolerated and safe in patients receiving MHD, but did not result in significant changes in laboratory biomarkers of inflammation or oxidative stress, improvements in serum lipid profiles, or in reductions in blood pressure. Future investigations are needed to test whether pomegranate juice–derived poly-phenols may be of benefit when administered for longer duration, at greater frequency or in certain patient subpopulations.

Practical Applications

The results from this pilot randomized crossover study show that pomegranate supplementation with either juice or extract tablets in patients undergoing MHD is safe, well tolerated, and feasible. Larger studies are needed to determine whether pomegranate supplementation leads to significant improvements in clinical outcomes in patients with ESRD.

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Supplementary Material

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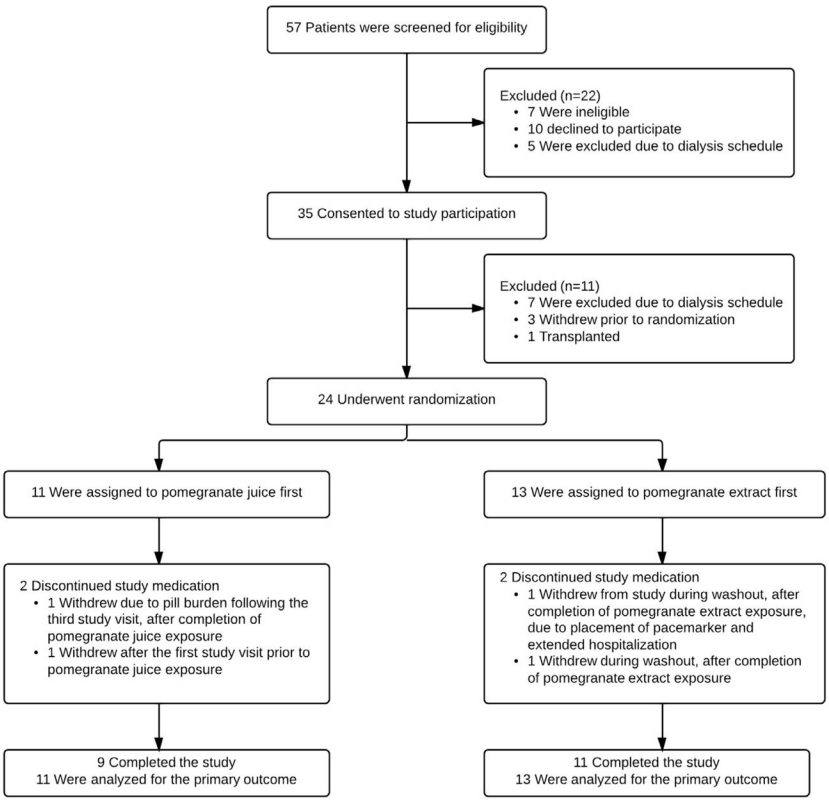

Figure 1.

Flow chart of study populations, including the number of patients who were screened, gave consent, underwent randomization, completed the study treatment and were analyzed for the primary outcome.

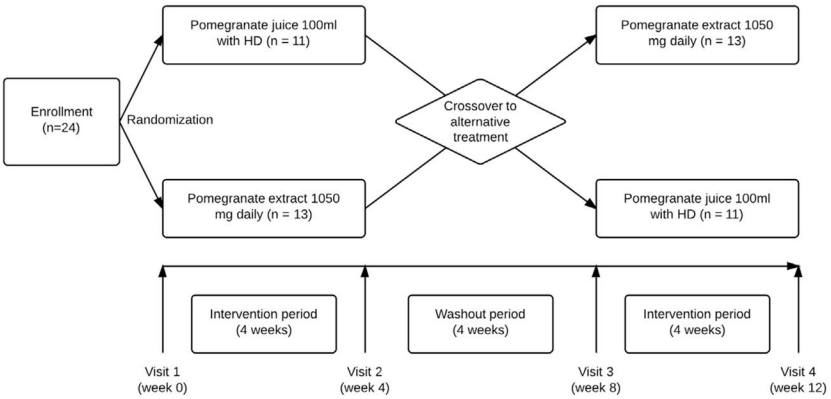

Figure 2.

Schematic diagram of study protocol showing sequential phases of the study, crossover, and study visits. HD, hemodialysis.

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Table 1

Baseline Patient Characteristics

	All Participants	Juice First	Extract First
N	24	11	13
Age, y, mean ± SD	61 ± 14	62 ± 10	59 ± 17
Male sex, *n* (%)	11 (46)	4 (36)	7 (54)
Height, cm, mean ± SD	168 ± 10	168 ± 8	168 ± 12
Weight, kg, mean ± SD	85 ± 24	80 ± 25	88 ± 24
Body mass index, kg/m^2, mean ± SD	30 ± 7	28 ± 8	31 ± 6
Current smoker, *n* (%)	3 (13)	2 (18)	1 (8)
Ever smoker, *n* (%)	13 (54)	6 (55)	7 (54)
Race, *n* (%)			
Asian	2 (8)	0 (0)	2 (15)
Black or African American	15 (63)	9 (82)	6 (46)
White	7 (29)	2 (18)	5 (38)
Access type, *n* (%)			
Fistula	14 (58)	6 (55)	8 (62)
Graft	10 (42)	5 (45)	5 (38)
Etiology of ESRD, *n* (%)			
Diabetes	8 (33)	2 (18)	6 (46)
Hypertension	8 (33)	5 (45)	3 (23)
Glomerulonephritis	3 (13)	1 (9)	2 (15)
Other/unknown	4 (17)	3 (27)	2 (16)
Dialysis vintage, *n* (%)			
3–6 mo	1 (4)	1 (9)	0 (0)
6–24 mo	8 (33)	5 (45)	3 (23)
2–5 y	8 (33)	3 (27)	5 (38)
>5 y	7 (29)	2 (18)	5 (38)
Comorbid conditions, *n* (%)			
Diabetes mellitus, type 1	2 (8)	0 (0)	2 (15)
Diabetes mellitus, type 2	8 (33)	4 (36)	4 (31)
Coronary artery disease	4 (17)	0 (0)	4 (31)
Hypertension	24 (100)	11 (100)	13 (100)
Peripheral vascular disease	4 (17)	1 (9)	3 (23)
Cancer	1 (4)	0 (0)	1 (8)
Medications, *n* (%)			
Erythrocyte stimulation agents (IV or SC)	16 (67)	9 (82)	7 (54)
Vitamin D supplements (IV or PO)	20 (83)	10 (91)	10 (77)
Iron supplements (IV or PO)	10 (42)	6 (55)	4 (31)
Cinacalcet	6 (25)	2 (18)	4 (31)
Phosphorous binders	19 (79)	9 (82)	10 (77)
Any antihypertensive	13 (54)	9 (82)	4 (31)

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	All Participants	Juice First	Extract First
ACEI/ARB	4 (17)	2 (18)	2 (15)
Beta blockers	7 (29)	5 (46)	2 (15)
Calcium channel blockers	7 (29)	6 (55)	1 (8)
Other antihypertensive	8 (33)	4 (36)	4 (31)
Insulin	7 (29)	2 (18)	5 (38)
Inhaled or oral corticosteroid	3 (13)	1 (9)	2 (15)

ESRD, end-stage renal disease; SD, standard deviation.

Values are number (percent), except as indicated otherwise.

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Table 2

Effects of Pomegranate Juice and Pomegranate Extract on Serum Lipids and Biomarkers of Inflammation and Oxidative Stress

	Juice			Extract		
	Pre	**Post**	***P* Value***	**Pre**	**Post**	***P* Value***
Total cholesterol (mg/dL)	166 (36)	172 (45)	.28	166 (41)	166 (36)	.91
HDL-C (mg/dL)	50 (17)	48 (17)	.30	50 (16)	50 (20)	.86
Triglycerides (mg/dL)	147 (74)	134 (67)	.28	150 (90)	156 (81)	.56
LDL-C (mg/dL)	88 (33)	97 (39)	.09	86 (34)	85 (32)	.81
C-reactive protein (mg/L)	7.5 (4.3, 11.5)	7.0 (2.5, 9.5)	.27	8.0 (6, 10)	7.5 (6, 10.5)	.63
IL-6 (pg/mL)	5.1 (2.7, 6.9)	7.9 (4.4, 9.2)	.40	6.3 (4.1, 9.4)	6.0 (4.1, 8.9)	.77
F_2-isoprostanes (pg/mL)	43.4 (16.5)	42.5 (17.6)	.65	45.4 (15.9)	42.5 (14.4)	.21
Isofurans (pg/mL)	88.8 (44.0)	97.6 (56.3)	.29	89.0 (59.3)	83.3 (46.5)	.27

HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin 6; LDL-C, low-density lipoprotein cholesterol.

Mean (standard deviation) values are presented for cholesterol, triglycerides, HDL-C, LDL-C, F_2-isoprostane, and isofurans. Medians (inter-quartile range) are presented for C-reactive protein and IL-6.

*P values obtained using paired t-tests of mean log-transformed values for IL-6 and C-reactive protein; P values for all other variables obtained using paired t-tests of mean values.

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Table 3

Comparison Effects of Pomegranate Juice and Pomegranate Extract on Serum Lipids and Biomarkers of Inflammation and Oxidative Stress

	Treatment Effect Estimate*	95% Confidence Interval	*P* Value†
Total cholesterol (mg/dL)	−3.8	−18.6 to 11.0	.61
HDL-C (mg/dL)	1.6	−3.6 to 6.9	.53
Triglycerides (mg/dL)	20.3	−8.5 to 49.1	.16
LDL-C (mg/dL)	−9.5	−23.1 to 4.2	.17
Log C-reactive protein (units)	0.25	−0.19 to 0.68	.26
Log IL-6 (units)	−0.15	−0.48 to 0.19	.38
F_2-isoprostanes (pg/mL)	−1.6	−7.5 to 4.2	.58
Isofurans (pg/mL)	−15.0	−33.0 to 3.0	.10

HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin 6; LDL-C, low-density lipoprotein cholesterol.

*Treatment effect estimate adjusted for baseline values for each outcome variable. Negative treatment effect estimates indicate lower values for outcome variables after treatment with pomegranate extract; positive treatment effect estimates indicate lower values after treatment with pomegranate juice.

†*P* values obtained using linear regression with adjustment for baseline values for each outcome variable.

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Table 4

Effects of Pomegranate Juice and Pomegranate Extract on Predialysis Blood Pressures

	Juice			Extract		
	Pre	**Post**	***P* Value***	**Pre**	**Post**	***P* Value***
Systolic blood pressure (mm Hg)	143 (23)	139 (22)	.16	140 (22)	137 (24)	.54
Diastolic blood pressure (mm Hg)	77 (14)	76 (13)	.50	77 (16)	74 (18)	.43
Mean arterial pressure (mm Hg)	99 (15)	97 (15)	.26	98 (17)	95 (19)	.46

mm Hg, millimeters of mercury.

Values are presented as mean (standard deviation).

* *P* values obtained using paired t-tests mean values.

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Table 5

Effects of Pomegranate Juice and Pomegranate Extract on Monocyte Cytokine Production, by Stimulus

		Juice			Extract		
Cytokine	**Stimulus**	**Pre**	**Post**	***P* Value**	**Pre**	**Post**	***P* Value**
IL-10 (pg/mL)	RPMI	10.6 (4.9–20.3)	10.5 (4.4–14.0)	.86	8.6 (6.5–18.5)	11.1 (4.1–15.1)	.74
	LPS	89.0 (65.9–196.2)	97.3 (83.4–200.4)	.75	124 (72.6–200.5)	83.8 (49.0–161.5)	.62
	Pam3Cys	30.6 (23.3–55.2)	30.3 (17.8–51.6)	.56	32.5 (20.2–48.1)	30.3 (18.1–41.9)	.78
	Flagellin	25.6 (21.0–42.0)	36.0 (15.4–43.9)	.80	33.4 (21.0–40.0)	30.6 (16.0–43.6)	.84
IL-1β (pg/mL)	RPMI	86.6 (26.1–210.6)	16.6 (4.8–56.6)	.005	42.3 (5.3–301.0)	93.9 (2.3–272.4)	.98
	LPS	764 (447–1,523.6)	845 (492.6–1,899.0)	.67	945 (487.3–1,409.3)	754 (392.0–1,591.2)	.80
	Pam3Cys	430 (245.6–680.4)	165 (115.7–367.4)	.03	325 (92.2–592.6)	182 (108.3–316.0)	.68
	Flagellin	274 (106.6–633.6)	206 (115.2–341.2)	.50	289 (153.2–640.1)	253 (124.4–319.8)	.91
IL-6 (ng/mL)	RPMI	2.6 (0.4–9.1)	1.5 (0.5–6.3)	.73	3.64 (1.2–9.2)	1.26 (0.5–17.3)	.66
	LPS	37.9 (28.6–56.4)	42.7 (31.6–54.6)	.91	40.8 (27.9–54.2)	39.1 (29.2–48.4)	.33
	Pam3Cys	23.1 (14.3–41.4)	27.4 (18.2–37.2)	.84	27.8 (16.3–37.4)	27.0 (20.2–31.8)	.61
	Flagellin	24.9 (11.7–31.2)	26.3 (17.3–36.8)	.10	25.7 (18.1–34.9)	26.2 (18.6–32.9)	.27
TNF-α (pg/mL)	RPMI	12.0 (5.4–22.3)	6.9 (2.3–29.8)	.46	11.7 (2.3–37.7)	12.5 (2.3–34.7)	.90
	LPS	103 (59.5–515.5)	390 (81.4–1,055.3)	.41	137 (75.7–241.9)	118 (78.2–304.1)	.56
	Pam3Cys	43.1 (30.7–87.8)	49.2 (29.2–188.1)	.97	39.9 (20.1–74.8)	43.4 (32.1–49.7)	.95
	Flagellin	46.5 (21.2–76.2)	59.9 (34.9–190.2)	.21	53.1 (40.5–64.6)	49.7 (38.7–63.9)	.37

IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-10, interleukin 10; LPS, lipopolysaccharide; Pam3Cys, (S)-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser(S)-Lys4-OH); RPMI, Roswell Park Memorial Institute medium; TNF-α, tumor necrosis factor alpha.

Values are presented as median (interquartile range).

* *P* values obtained from paired t-tests of mean log-transformed values.

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Table 6

Comparison Effects of Pomegranate Juice and Pomegranate Extract on Monocyte Cytokine Production, by Stimulus

	Stimulus	Treatment Effect Estimate*	95% Confidence Interval	*P* Value†
Log IL-10	RPMI	−0.014	−0.62 to 0.60	.96
	LPS	−0.26	−0.81 to 0.29	.34
	Pam3Cys	−0.15	−0.68 to 0.38	.57
	Flagellin	0.007	−0.47 to 0.49	.98
Log IL-1β	RPMI	0.80	−0.45 to 2.04	.20
	LPS	−0.11	−0.76 to 0.54	.73
	Pam3Cys	−0.13	−0.71 to 0.44	.64
	Flagellin	−0.04	−0.44 to 0.52	.85
Log IL-6	RPMI	0.15	−1.03 to 1.34	.79
	LPS	−0.06	−0.42 to 0.29	.72
	Pam3Cys	−0.19	−0.76 to 0.38	.50
	Flagellin	0.06	−0.36 to 0.49	.76
Log TNF-α	RPMI	0.26	−0.72 to 1.24	.60
	LPS	−0.55	−1.42 to 0.32	.21
	Pam3Cys	−0.44	−1.14 to 0.26	.21
	Flagellin	−0.22	−0.84 to 0.41	.49

IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-10, interleukin 10; LPS, lipopolysaccharide; Pam3Cys, (S)-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser(S)-Lys4-OH); RPMI, Roswell Park Memorial Institute medium; TNF-α, tumor necrosis factor alpha.

*Treatment effect estimate adjusted for baseline values for each outcome variable, presented as log unit differences. Negative treatment effect estimates indicate lower values for outcome variables after treatment with pomegranate extract; positive treatment effect estimates indicate lower values after treatment with pomegranate juice.

†*P* values obtained using linear regression with adjustment for baseline values for each outcome variable.