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CLINICAL RESEARCH STUDY

Low-level Environmental Exposure to Lead and Progressive Chronic Kidney Diseases

Ja-Liang Lin, MD, Dan-Tzu Lin-Tan, RN, Yi-Jung Li, MD, Kuan-Hsing Chen, MD, Yen-Lin Huang

Department of Nephrology and Division of Clinical Toxicology, Chang Gung Memorial Hospital, Lin-Kou Medical Center, School of Medicine, Chang Gung University, Taipei, Taiwan, ROC.

ABSTRACT

PURPOSE: To determine whether low-normal body lead burden (BLB) accelerates progressive renal insufficiency in nondiabetic patients with chronic kidney diseases (CKD).

METHODS: One hundred eight CKD patients (serum creatinine between 1.5 and 2.9 mg/dL) with low-normal BLB (<80 μg) and no lead exposure history were observed for 24 months. Following the observation, 32 patients with low-normal BLB ($\geq$20 μg and <80 μg) were randomly assigned to chelation and control groups. The chelation group patients were given edetate calcium disodium (EDTA) chelation therapy for 3 months and repeated chelation therapy during the following 24 months to maintain their BLB below 20 μg, while the control group patients underwent placebo therapy. The primary endpoint was an increased serum creatinine level to 1.25 times the baseline value. The secondary endpoint was temporal changes in renal function.

RESULTS: The primary endpoint occurred in 14 patients in the observation period. Baseline BLB was the important risk factor in determining progressive renal insufficiency. The mean glomerular filtration rate (GFR) change in the chelation group patients was 6.6 ± 10.7 mL/min/1.73m^2, compared with -4.6 ± 4.3 mL/min/1.73m^2 in control group patients ($P < .001$) at the end of the intervention period. The mean decrease in GFR per year of chelation group patients was lower than that of control group patients during the repeated chelation period.

CONCLUSION: Environmental exposure to lead, even at low level, may accelerate progressive renal insufficiency of nondiabetic patients with CKD.

KEYWORDS: Progressive renal insufficiency; Repeated lead chelation therapy; Low-level environmental lead exposure; Nondiabetic chronic kidney diseases

Chronic lead nephropathy is related to long-term heavy lead exposure.[1-4] However, previous investigations[5-7] revealed blood lead levels (BLL) related to renal function and accelerated age-related renal function impairment in the general population.[8] BLL only indicates recent lead exposure whereas X-ray fluorescence and edetate calcium disodium (EDTA) mobilization tests are only reliable methods to assess body lead burdens (BLB). Some studies[9-15] used EDTA mobilization tests to evaluate BLB of nondiabetic patients with chronic kidney diseases (CKD) and demonstrated that normal BLB (<600 μg) was associated with progressive renal insufficiency in these patients.[8,10,15] Additionally, repeated lead chelation therapy may improve renal function, thus slowing progressive renal insufficiency in patients with high-normal BLB (>80 and <600 μg).[10,15] However, whether lead-related progressive renal insufficiency happens or is treatable by lead chelation therapy in CKD patients with low-normal BLB (<80 μg) is unclear.

Nevertheless, recent rapid falls in mean BLL have occurred in many countries.[16] Because low-level environmental lead exposure will become normal for the general population, it is important to know whether exposure to low-level lead affects cases such as progressive renal im-

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Requests for reprints should be addressed to Ja-Liang Lin, MD, Division of Nephrology and Clinical Toxicology, Chang Gung Memorial Hospital, 199 Tung Hwa North Road, Taipei, Taiwan, ROC.

E-mail address: jllin99@hotmail.com

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pairment in CKD patients. To test the above hypotheses, we performed this prospective longitudinal study accompanying a clinical trial.

METHODS

Subjects

The Medical Ethics Committee of Chang Gung Memorial Hospital approved the protocol, and all patients gave written informed consents.

Patients with CKD were selected if they were aged between 18 and 80 years old, and if their serum creatinine concentration was between 1.5 and 2.9 mg/dL with a decrease in the estimated glomerular filtration rate (GFR) of <5 mL/ minute/1.73m^2 over a period of at least 6 months. They also had blood pressure below 140/90 mm Hg, a cholesterol level lower than 240 mg/dL, a daily protein intake below 1 g/kg body weight, no known history of exposure to lead or other heavy metals, and BLB <80 μg measured by EDTA mobilization testing and 72-hour urine collection. Renal diagnoses were based on patient history, laboratory evaluations, renal imaging, and renal histological examinations.[17]

The exclusion criteria were renal insufficiency with a potentially reversible cause, such as malignant hypertension, urinary tract infection, hypercalcemia or drug-induced nephrotoxic effects; systemic diseases, such as malignancy, heart disease, connective-tissue diseases or diabetes mellitus; drug intakes that could change the progression of renal diseases, such as aristolochic-acid-containing herbs, nonsteroidal anti-inflammatory drugs (NSAID), steroids or immunosuppressive drugs; rapidly progressive glomerulonephritis or very high 24-hour urinary protein (more than 8 g per day); drug allergies; and lack of informed consents.

Diuretics and angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor antagonists (ARA), with or without nondihydropyridine calcium-blocking agents, provided blood pressure control. Patients with normal blood pressure received neither ACE inhibitors nor ARA. Antihypertension drugs were not changed throughout the study. Blood pressure, cholesterol, and protein intake were effectively controlled in all patients, while phosphate levels were managed with calcium carbonate. No patients received vitamin D3 supplements or erythropoietin. Dietary consultations were given, recommending a normal-protein diet (0.8 to 1.0 g/kg per day). A nutritionist reviewed each patient's diet every 6 months, and 24-hour urea excretion was measured every 3 months to determine nitrogen balance and dietary compliance.[18]

CLINICAL SIGNIFICANCE

- Environmental exposure to lead, even at low-level, may accelerate progressive renal insufficiency of non-diabetic patients with chronic kidney diseases.
- Repeated chelation therapy may slow the progression in the long term in these patients.

Measurement of BLL and BLB

BLL and BLB were measured as described.[9-15] BLB measurement used EDTA-mobilization tests developed by Emmerson and modified by Behringer et al.[19] BLB were assessed using 72-hour urinary lead excretion following intravenous infusion of 1 g of calcium disodium EDTA (Calcium Disodium Versenate, Abbott Laboratories, North Chicago, IL). BLL and BLB were measured by electrothermal atomic-absorption spectrometry (model 5100 PC, Perkin-Elmer, Boston, MA) with a Zeeman background correction and an L'vov platform. Throughout this study, both internal and external quality-control procedures were used, with consistently satisfactory results.[15] Low-normal BLB was defined as below 80 μg in previous studies.

STUDY PROTOCOL

Baseline Data Collection Period (Months −9- 0)

Of 300 chronic renal disease cases screened, 124 met the inclusion criteria and joined this study. Baseline BLL, hemoglobin levels and BLB were measured before the study. Serum creatinine, blood urea nitrogen, cholesterol, urinary protein, creatinine and urea excretion, were all determined every 3 months with an auto-analyzer system (Hitachi) to ensure entry criteria compliance. Blood pressure and body weight were also measured at every assessment.

The Observation Period (Months 0-24)

Serum creatinine, blood urea nitrogen, cholesterol, urinary protein, creatinine and urea excretion were determined every 3 months from month 0 to month 51 in all study patients. Urinary excretion measurements were averaged values from 2 consecutive 24-hour urine collections, while renal function evaluation was based on creatinine clearance (Ccr) and estimated GFR.[20]

The Intervention Period (Months 24-51)

The observational study was followed by a single-blind, randomized, placebo-controlled 27-month investigation, in which all patients were randomly assigned either placebo or chelation therapy in the initial 3 months, then received placebo or repeated chelation therapy, if required, over the next 24 months. Mobilization tests were performed again to ensure that the BLB of all patients was less than 80 μg. The lead chelation therapy was held if the BLB fell below 20 μg, which was equal to the means BLB (40.2 μg) minus one standard deviation (21.2 μg) of all study subjects. Patients whose GFR had not decreased by the end of the 24-month observation period were excluded.

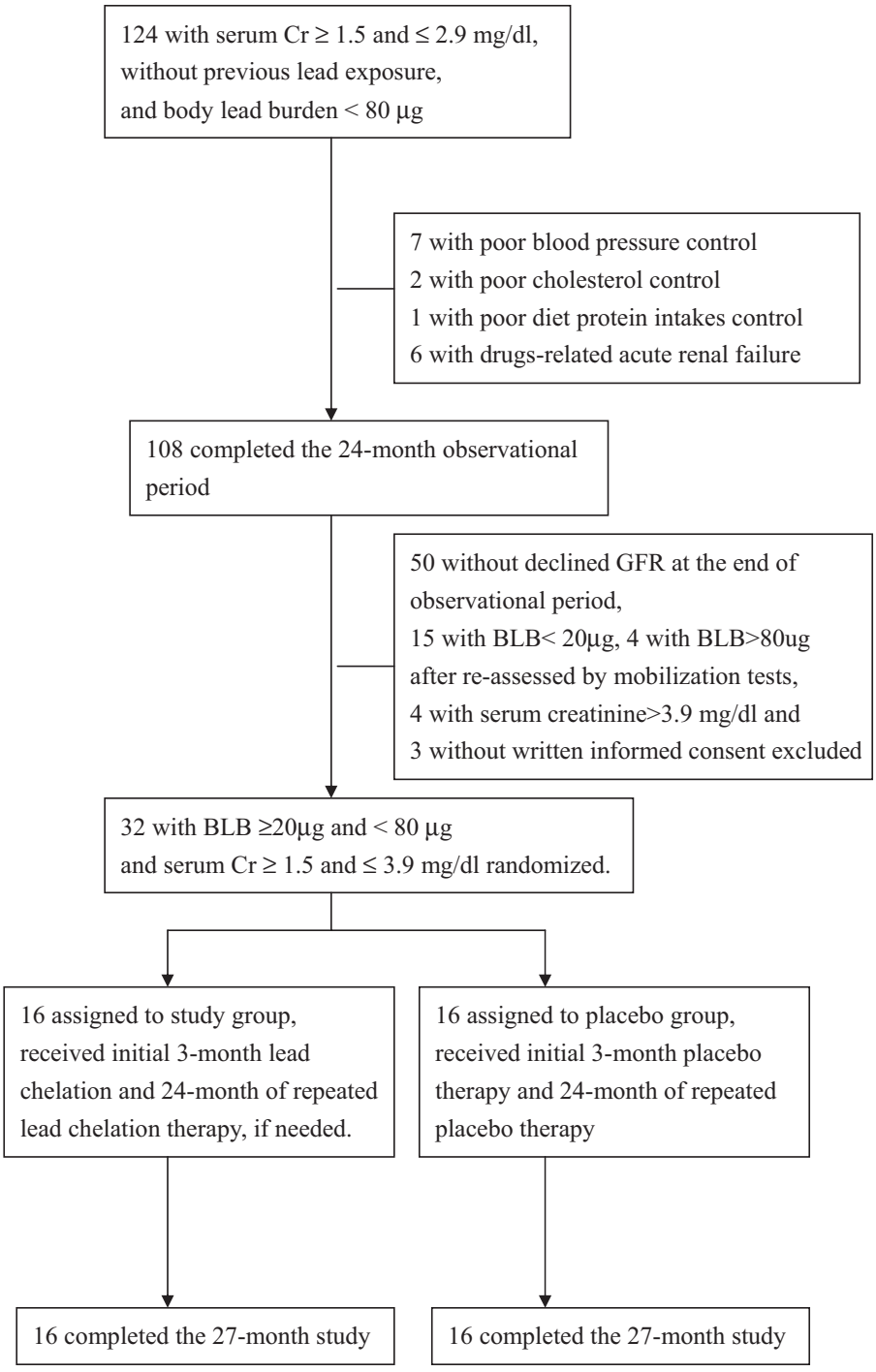

Figure 1 Diagram showing the flow of patients.

Initial Chelation Therapy (Months 24-27)

Among 108 patients completing the observation period, 32 with low normal BLB (20 μg ≤ and <80 μg), progressively declining GFR during the observation period, and serum creatinine <4.0 mg/dL were randomly assigned to the control or chelation groups in a proportion of 1:1. Control group patients received weekly intravenous infusion of one placebo vial (20 mL) of 50% glucose, mixed with 200 mL normal saline over 2 hours for 5 weeks.[15] Meanwhile, chelation group patients received weekly intravenous infusion of 1 vial (1 g) of calcium di-sodium EDTA mixed with 200 mL normal saline over 2 hours except when the BLB fell below 20 μg. Because the drugs were pharmacy-prepared, patients were unaware which ones they received.

Repeated Chelation Period (Months 27- 51)

If, on rechecking 1 month later, study group patients increased their serum creatinine above prechelation levels (the baseline levels at the beginning of the intervention period) and their BLB exceeded 20 μg, or if their BLB was above

Table 1 Cox Regression Analysis of the Overall Risk of the Primary Outcome of Progressive Renal Insufficiency, According to Baseline Prognostic Factors

Variable	Hazard Ratio (95% CI)*	*P* Value
Age (each increment of 1 year)	1.02 (0.97-1.07)	.3688
Female sex	7.72 (1.96-30.42)	.0035
Baseline body mass index (each increment of 1)	0.94 (0.82-1.08)	.3671
Smoking	3.75 (0.36-40.00)	.2715
Baseline serum creatinine (each increment of 1mg/dL [88 μmol/L])	1.63 (0.26-10.35)	.6019
Body lead burden (each increment of 1 μg)	1.03 (1.00-1.07)	.0375
Baseline daily protein excretion (each increment of 1 g)	0.68 (0.30-1.51)	.3392
Hypertension	1.34 (0.17-5.56)	.1248
Hyperlipidemia	1.18 (0.28-4.95)	.8244
Baseline daily protein intake (each increment of 1 g/kg)	2.06 (0.19-22.8)	.5560
Chronic glomerulonephritis	1.26 (0.40-4.02)	.3879

*CI denotes confidence interval. The body mass index is the weight in kilograms divided by the square of the height in meters. The primary endpoint was defined as an increase in the serum creatinine level to 1.25 times the baseline value during the observation period.

20 μg at 6 monthly BLB reassessments during this period, repeating EDTA chelation therapy would begin 1 g EDTA intravenous injection weekly until their BLB fell below 20 μg again. The control group received placebo therapy weekly for 5 weeks every 6 months. At the end of this period, the renal function changes were compared.

Study Compliance

Patients were excluded from the study if they did not complete it, developed poorly controlled hypertension (>160/95 mm Hg), hyperlipidemia (cholesterol >260 mg/dL), a protein intake exceeding 1.5 g/kg per day for more than 6 months, or acutely deteriorating renal function secondarily to drugs or other etiologies such as trauma, hyperthermia, NSAIDs, or herbs during the study period.

Outcome Measures

The primary endpoint was an increase in serum creatinine to 1.25 times baseline levels, measured on 2 occasions 1 month apart, or the requirement for renal replacement therapy during the observation period. The secondary endpoint was temporal changes in Ccr or GFR values during the intervention period.

Statistical Analysis

Sample size was calculated with PASS software (power analysis and sample-size package, NCSS statistical software). For a 2-sided test at the .05 significance level, a 32-patient sample (16 per group) would permit a detection of difference between groups in the change rate of GFR of 0.31 mL per minute per 3-month interval,[9] with a power of >.82. The influence of variables[21] in predicting the primary endpoint during the observation period was determined with the Cox proportional-hazards model. Additionally, a general estimation equation (GEE) was applied to longitudinal multivariate analyses using statistical software, SAS 6.12 (SAS Institute Inc., Cary, NC). The progressive renal insufficiencies of the 2 groups were compared during the study period, with the chi-squared test, Student's *t* test and Mann-Whitney *U* test measuring the differences between them. All *P* values were 2-tailed, and all results were represented by the mean ±SD (standard deviation).

RESULTS

The Observation Period (Months 0-24)

Of the initial 124 patients (110 men, 14 women), 108 finished the 24-month observation period (Figure 1). At the baseline, the patients' mean age was 56.2 ± 12.7 years (range 30-80); their body mass index (kg/m^2) was 25.6 ± 3.6 (range 18.2-35.0); their serum creatinine level was 1.8 ± 0.3 mg/dL (range 1.5-2.8); the Ccr was 50.2 ± 12.8 mL/min/1.73 m^2 (range 25.2 to 88.3); the GFR was 47.6 ± 9.8 mL/min/1.73 m^2 (range 25.7-69.4); the daily protein excretion was 0.92 ± 0.85 g (range 0.05-6.20); the daily protein intake was 0.91 ± 0.24 g/kg (range 0.29-1.73); the BLL was 2.9 ± 1.4 μg/dL (range 0.8-10.3); and the BLB was 40.2 ± 21.2 μg (range 1.7 to 78). Twenty-two patients (20.3%) had hyperlipidemia. Seventy-eight patients (72.2%) suffering from hypertension were treated with ACE inhibitors, or ARA in 71 patients (65.7%). Six patients (5.6%) smoked.

Underlying renal diseases comprised of chronic glomerulonephritis in 42 patients (38.9%), chronic interstitial nephritis in 20 (18.5%), hypertensive nephropathy in 17 (15.7%), polycystic kidney disease in 8 (7.4%), obstructive uropathy in 5 (4.6%), and unknown diseases in 16 (14.8%). At the end of the observational period, the serum creatinine level was 1.9 ± 0.4 mg/dL (range 1.5-4.0), the Ccr was 47.1 ± 12.5 mL/min/1.73 m^2 (range 23.6-88.3), and the estimated GFR was 44.3 ± 10.9 mL/min/1.73 m^2 (range 18.8-70.6).

While 14 patients achieved the primary outcome without requiring renal replacement therapy, none of these had a BLB less than 20 μg by the end of the observation period. Cox multivariate analysis indicated female sex and BLB as important risk factors (Table 1). Longitudinal analysis of all patients using GEE revealed basal BLB and daily protein intake as

Table 2 Longitudinal Analysis of Body Lead Burden and other Predictors of Progressive Change in the Glomerular Filtration Rate, Using Generalized Estimating Equations, during the 24-Month Longitudinal Study Period

Variable	Estimate (Interactive effect)*	*P* Value
Age (each increment of 1 year)	−0.0712	.1471
Sex (male vs. female)	−3.4351	.0922
Body mass index (each increment of 1)	0.1553	.3413
Hyperlipidemia (yes vs. no)	0.5173	.7388
Hypertension (yes vs. no)	−3.0627	.1501
Smoking (yes vs. no)	0.1752	.9448
Baseline serum creatinine (each increment of 1 mg/dL [88 μmol/L])	−2.9533	.1225
Body lead burden (each increment of 1 μg)	−0.1037	.0083
Daily protein excretion (each increment of 1 g)	−0.8704	.2616
Daily protein intake (each increment of 1 g/kg)	−5.2141	.0328
Underlying disease		
Chronic glomerulonephritis	2.3640	.2269
Chronic interstitial nephritis	3.6025	.0668
Hypertensive nephropathy	2.8550	.2105
Polycystic kidney disease	0.3019	.9716
Obstructive uropathy	5.4850	.0879
Unknown†	—	—

*The interactive effect of variables was calculated by a generalized estimating equation. Negative values for the interactive effect indicate a decline in the glomerular filtration rate, and positive values indicate an increase.

†The unknown group was the reference group.

significant predictors of progressive GFR after adjusting for other factors (Table 2). Each 1-μg increase in BLB led to a 0.1037 mL/min/1.73m^2 reduction in GFR during the 2-year observation period. Fifty patients (46.3%) showed no reduced GFR after the 24 months of observation. The post hoc analysis showed that BLB was the only significant difference between the patients with and without reduced renal function (46.2 ± 18.3 μg vs 33.3 ± 22.3 μg, $P = .0013$).

The Intervention Period (Months 24-51)

Initial chelation period (months 24-27). Thirty-two patients with low-normal BLB (≥20 μg and <80 μg)

Table 3 Baseline Characteristics of Study Patients with Low Normal Body Lead Burden (≥20 ug and <80 ug) at the Entry of Clinical Trial

Variables	Study Group (n = 16)	Control Group (n = 16)	*P*
Age (Y/O)	58.6 ± 10.9 (48-74)	54.8 ± 13.1 (31-76)	.3854
Sex (M/F)	14/2	3/3	.9999*
Body mass index (kg/m^2)	25.4 ± 4.0 (18.4-33.7)	26.2 ± 4.0 (20.3-34.4)	.5825
Smoking	2	1	.9999*
Serum Cr (mg/dL)	2.1 ± 0.7 (1.6-3.7)	2.0 ± 0.5 (1.6-3.5)	.5892
Creatinine clearance (mL/min/1.73m^2)	41.2 ± 11.5 (24.2-69.0)	47.3 ± 15.4 (27.3-77.7)	.2142
Glomerular filtration rate (mL/min/1.73m^2)	41.2 ± 11.2 (19.0-54.8)	42.6 ± 9.7 (21.9-51.3)	.7653
Blood lead levels (ug/dL)	2.6 ± 1.0 (1.4-4.4)	3.0 ± 1.1 (1.2-4.6)	.3626
Body lead burden (ug)	43.1 ± 13.7 (20.0-72.4)	47.1 ± 15.8 (20.0-79)	.4425
Daily proteinuria (g/day)	1.04 + 1.21 (0.10-4.20	1.06 + 1.62 (0.10-6.20)	.8651†
Daily Protein intake (g/kg)	1.02 ± 0.26 (0.8-1.7)	0.93 ± 0.21 (0.6-1.3)	.2675
Hyperlipidemia	6	4	.7043*
Hypertension	13	13	.9999*
Using CEI or ARA in hypertension	13	11	.6851*
Using dihydropyridine CCB	8	6	.7216*
Underlying renal disease			
Chronic glomerulonephritis	6	7	.9999*
Chronic interstitial nephritis	5	5	.9999*
Hypertensive nephropathy	2	1	.9999*
Unknown	3	3	.9999*

Hyperlipidemia: serum cholesterol >240 mg/dL after diet control; Hypertension: blood pressure >140/90 mm Hg at least twice measurements and with anti-hypertensive drugs. CEI = converting enzyme inhibitors; ANA = angiotensin II receptor antagonists; CCB = calcium channel blockers.

*Data were measured by the chi-squared with Fisher's exact test.

†Data by Mann-Whitney *U* method, and other data by the Student *t* test.

Table 4 The Means of Body Mass Index, Mean Arterial Pressure, Serum Cholesterol, Daily Urine Protein and Daily Protein Intake in Study Patients during the 51-Month Study Period

Variables	Study Group (n = 16)	Control Group (n = 16)	*P*
Month 0			
Body mass index (kg/m^2)	25.2 ± 3.6	26.2 ± 3.5	.4164
MAP (mm Hg)	96.2 ± 8.2	96.6 ± 8.6	.8952
Cholesterol (mg/dL)	205.8 ± 37.8	199.3 ± 35.8	.6341
24-h urine protein (g)	0.88 ± 1.17	1.14 ± 1.68	.5211*
Daily Protein intake (g/kg)	1.07 ± 0.24	0.99 ± 0.20	.2961
Month 6			
Body mass index (kg/m^2)	25.2 ± 3.6	25.9 ± 3.6	.5388
MAP (mm Hg)	97.2 ± 9.4	95.0 ± 10.6	.5455
Cholesterol (mg/dL)	204.6 ± 41.7	197.8 ± 29.8	.5993
24-h urine protein (g)	0.77 ± 0.98	1.03 ± 1.34	.4617*
Daily protein intake (g/kg)	1.01 ± 0.16	1.02 ± 0.21	.8899
Month 12			
Body mass index (kg/m^2)	25.2 ± 4.0	25.3 ± 3.9	.4384
MAP (mm Hg)	92.7 ± 9.4	96.5 ± 10.4	.3444
Cholesterol (mg/dL)	193.8 ± 33.8	194.9 ± 35.9	.9239
24-h urine protein (g)	0.82 ± 0.78	1.02 ± 1.05	.8356*
Daily protein intake (g/kg)	1.04 ± 0.20	0.95 ± 0.23	.3023
Month 18			
Body mass index (kg/m^2)	25.2 ± 3.7	26.1 ± 3.9	.4901
MAP (mm Hg)	93.3 ± 6.7	96.5 ± 10.4	.3083
Cholesterol (mg/dL)	193.6 ± 33.2	203.2 ± 37.5	.4509
24-h urine protein (g)	0.90 ± 0.94	1.16 ± 1.66	.7628*
Daily protein intake (g/kg)	0.97 ± 0.20	0.96 ± 0.20	.8899
Month 24			
Body mass index (kg/m^2)	25.4 ± 4.0	26.2 ± 4.0	.5825
MAP (mm Hg)	94.1 ± 6.6	96.6 ± 8.6	.3615
Cholesterol (mg/dL)	194.9 ± 41.9	202.1 ± 30.3	.5951
24-h urine protein (g)	1.04 ± 1.21	1.06 ± 1.62	.8651*
Daily protein intake (g/kg)	1.02 ± 0.26	0.93 ± 0.21	.2675
Month 27			
Body mass index (kg/m^2)	25.2 ± 3.9	26.1 ± 4.0	.5091
MAP (mm Hg)	91.4 ± 8.4	95.7 ± 7.8	.1429
Cholesterol (mg/dL)	193.9 ± 28.9	201.4 ± 38.6	.5381
24-h urine protein (g)	0.92 ± 1.16	1.11 ± 1.63	.6236*
Daily protein intake (g/kg)	1.04 ± 0.24	0.96 ± 0.18	.3070
Month 33			
Body mass index (kg/m^2)	25.3 ± 3.8	26.2 ± 4.0	.5341
MAP (mm Hg)	92.8 ± 8.4	95.2 ± 11.7	.4959
Cholesterol (mg/dL)	200.3 ± 30.4	210.5 ± 40.3	.4259
24-h urine protein (g)	0.80 ± 0.87	1.02 ± 1.32	.4284*
Daily protein intake (g/kg)	0.99 ± 0.24	0.97 ± 0.17	.7809
Month 39			
Body mass index (kg/m^2)	25.3 ± 3.8	26.2 ± 3.9	.5091
MAP (mm Hg)	92.2 ± 8.5	95.9 ± 9.9	.2562
Cholesterol (mg/dL)	202.1 ± 38.6	204.8 ± 29.4	.8221
24-h urine protein (g)	0.91 ± 1.01	1.12 ± 1.59	.6508*
Daily protein intake (g/kg)	0.95 ± 0.18	1.00 ± 0.18	.4485
Month 45			
Body mass index (kg/m^2)	25.4 ± 3.4	26.3 ± 3.9	.4817
MAP (mm Hg)	91.6 ± 7.1	95.3 ± 3.4	.2062
Cholesterol (mg/dL)	198.3 ± 35.5	209.9 ± 35.9	.3615
24-h urine protein (g)	1.11 ± 1.30	1.00 ± 1.21	.7917*
Daily protein intake (g/kg)	0.96 ± 0.18	0.93 ± 0.18	.6453
Month 51			
Body mass index (kg/m^2)	25.1 ± 3.5	26.4 ± 3.9	.3578
MAP (mm Hg)	92.4 ± 8.5	96.0 ± 8.2	.2208
Cholesterol (mg/dL)	199.1 ± 36.5	212.9 ± 34.5	.2393
24-h urine protein (g)	0.90 ± 0.88	1.08 ± 1.27	.8502*
Daily protein intake (g/kg)	1.02 ± 0.24	0.93 ± 0.15	.2593

MAP = mean arterial pressure. Data were measured by the Student's *t* test except *data by Mann-Whitney *U* method.

Table 5 The Means of Renal Function during the 51-Month of Study Period

Renal Function (ml/min/1.73m^2)	The Study Group (n = 16)	The Control Group (n = 16)	*P* (95% CI)
Basal observation period (Month 0-Month 24)			
Month 0			
Ccr	48.6 ± 9.2	52.2 ± 16.3	.6540 (−13.2-5.9)
GFR	49.9 ± 7.8	48.7 ± 10.3	.7091 (−5.4-7.8)
Month 6			
Ccr	46.4 ± 9.0	49.2 ± 16.2	.5476 (−12.7-6.6)
GFR	47.2 ± 8.6	45.1 ± 10.6	.5492 (−4.9-9.1)
Month 12			
Ccr	44.5 ± 9.2	49.3 ± 16.1	.3146 (−14.2-4.7)
GFR	45.1 ± 10.5	45.0 ± 11.0	.9724 (−7.6-7.9)
Month 18			
Ccr	45.0 ± 8.4	48.8 ± 16.9	.4274 (−13.4-5.8)
GFR	45.0 ± 9.0	44.3 ± 11.4	.8423 (−6.7-8.1)
Month 24			
Ccr	41.2 ± 11.5	47.3 ± 15.4	.2142 (−15.9-3.7)
GFR	41.2 ± 11.2	42.6 ± 9.7	.7253 (−8.9-6.3)
Rate of decrease of renal function (mL/min/year) during the observation period			
Ccr	3.7 ± 3.2	2.4 ± 2.9	.2000 (−1.0-3.4)*
GFR	4.3 ± 3.3	3.1 ± 1.4	.3271 (−.6-3.1)*
Initial lead chelation period (Month 24-Month 27)			
Increase renal function (ml/min) after initial 3-month lead chelation therapy			
Ccr	5.9 ± 4.7	1.8 ± 2.3	.0064 (1.5-6.9)*
GFR	6.8 ± 5.9	2.2 ± 2.4	.0143 (1.4-7.9)*
Repeat lead chelation period (Month 27-Month 51)			
Month 27			
Ccr	47.2 ± 14.0	49.1 ± 14.9	.7103 (−12.3-8.5)
GFR	48.1 ± 14.0	44.7 ± 8.8	.4235 (−5.1-11.8)
Month 33			
Ccr	46.8 ± 12.2	47.8 ± 14.9	.8460 (−10.8-8.9)
GFR	47.7 ± 14.2	43.6 ± 9.0	.3363 (−4.5-12.7)
Month 39			
Ccr	46.2 ± 10.5	46.8 ± 15.9	.8982 (−10.3-9.1)
GFR	47.5 ± 12.2	42.6 ± 9.2	.2087 (−2.9-12.7)
Month 45			
Ccr	45.3 ± 11.3	44.1 ± 13.6	.7883 (−7.8-10.2)
GFR	47.2 ± 14.6	39.7 ± 9.7	.0986 (−1.6-16.4)
Month 51			
Ccr	46.6 ± 12.8	42.2 ± 13.2	.3537 (−5.1-13.8)
GFR	47.9 ± 17.0	38.0 ± 8.9	.0483 (.1-19.7)
Rate of decrease of renal function (mL/min/year) during the repeat chelation period			
Ccr	0.29 ± 4.3	3.4 ± 1.8	.0176 (.7-5.5)*
GFR	0.11 ± 5.4	3.4 ± 1.8	.0458 (.3-6.2)*
Increments of renal function at the end of clinical trial			
Ccr	5.3 ± 6.5	−5.1 ± 4.1	<.0001 (6.5-14.3)*
GFR	6.6 ± 10.7	−4.6 ± 4.3	.0005 (5.3-17.1)*

Data were measured by the Student's *t* test except *data by Mann-Whitney *U* method. $P < .05$ means significant differences.

participated in the clinical trial and were randomly divided into chelation and control groups. Both groups displayed similar basic characteristics (Table 3), and following 3 months of lead chelation therapy, the BLB of the study group patients decreased to 11.8 ± 6.5 μg (range 5-19.2), while BLL fell to 1.9 ± 0.7 μg/dL (range 1.2-2.9). The therapeutic EDTA dosages averaged 3.1 ± 0.7 g (range 2-4). Meanwhile, the improved renal function (GFR) of the study group (6.8 ± 5.9 mL/min/1.73m^2) exceeded that of the control group (2.2 ± 2.4 mL/min/1.73m^2, $P = .0143$ in Mann Whitney *U* test).

Repeated chelation period (months 27-51). No significant differences appeared between the 2 groups in body mass index, mean arterial pressure, cholesterol, daily urine proteinuria, and daily protein intake during this period (Table 4). Improved renal function in chelation group patients extended for at least 24 months (Table 5) (Figure 2), while

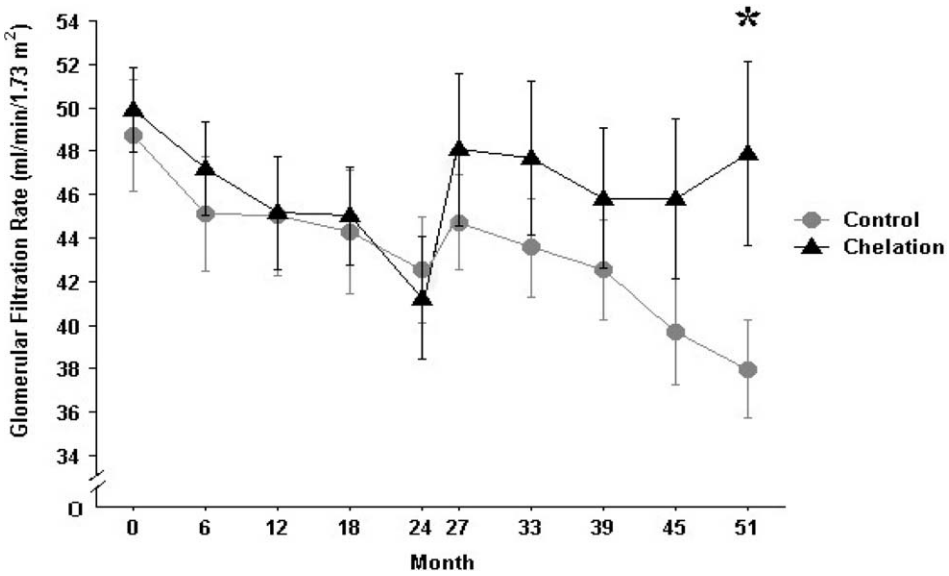

Figure 2 Estimated mean (± SE) glomerular filtration rate according to time in the chelation group and the control group during the observation and intervention period. The patients in the chelation group received chelation therapy from month 24 to month 51. The asterisk indicates $P < .05$ by Student's t test.

their progressive renal insufficiency was slower than that in the control group patients.

Nine study group patients (56.3 %) required repeated chelation therapy as their serum creatinine levels rose above prechelation therapy levels or their BLB also exceeded 20 μg. These patients' BLB rose to 49.9 ± 9.9 μg (range 28-62), and the mean EDTA dose for repeated chelation therapy was 3.0 ± 1.0 g (range 2-4). The average time between initial and subsequent chelation therapy was 16.0 ± 6.0 months (range 6-21), and the mean BLB after repeated chelation therapy was 10.5 ± 7.6 μg (range 0-18.2). All patients experienced reduced serum creatinine levels (prerepeated chelation creatinine: 1.9 ± 0.5 mg/dL; postrepeated chelation creatinine: 1.7 ± 0.3 mg/dL, $P = .0432$ in the paired t test) and improved renal function (prerepeated chelation GFR: 44.4 ± 11.0 mL/min/1.73m^2; postrepeated chelation GFR: 49.3 ± 11.9 mL/min/1.73m^2, $P < .0001$) after repeated chelation therapy.

DISCUSSION

Both Cox and longitudinal multivariate analysis demonstrate that BLB remains the only crucial risk factor in the progression of renal disease, even adjusting for other influences. Additionally, repeated lead chelation therapy improves renal function while slowing progressive renal insufficiency in these patients. These findings imply that chronic low-level environmental exposure to lead may subtly affect progressive renal insufficiency in patients with nondiabetic CKD.

While 14 patients achieved the primary outcome, none of them had a BLB <20 μg. Additionally, 46.3% patients (50/108) with no reduced GFR had a significantly lower basal BLB than those with reduced GFR at the end of the observation period. Such findings indicate BLB is an important influence on progressive renal insufficiency in nondiabetic CKD patients with low-level environmental exposure to lead. Moreover, no secure limits of environmental lead exposure are observed to precipitate progressing renal insufficiency in these patients. Each 10-μg BLB increase leads to a reduction of 1.037 mL/min/1.73 m^2 of GFR in the observational period. The mean BLL was only 2.9 μg/dL, resembling that (2.8 μg/dL) of the general American population after removal of possible lead sources from life, including leaded gasoline and leaded food cans,[16] whereas the mean BLB was 40.2 μg—far below the upper limit of the normal range (BLL, 20 μg/dL; BLB, 600 μg). These findings demonstrated that even low-level environmental exposure to lead may be unsafe and should not be ignored in patients with nondiabetic CKD.

Whether raised BLB represents a cause or consequence of CKD has been much debated.[22] However, most studies suggest that BLL or BLB causes rather than results from decreased renal function.[23-25] Chemical and histological studies of trans-iliac biopsies from 153 dialysis patients demonstrated that chronic renal failure does not cause lead to accumulate in bone.[26]

Finally, this work demonstrates that treatment may be possible for low-level lead-related progression of CKD. The mean BLB of study group patients reduced from 43.1 ug to 11.8 ug following initial chelation therapy, whereas their GFR increased by 16.5%. Additionally, the mean rates of decrease GFR (0.11 ± 5.4 mL/min/1.73m^2/year) of the chelation group was lower than that (3.4 ± 1.8 mL/min/1.73m^2/year, $P = .0458$) of the control group, although their GFR decreased similarly during the observation period. Both levels of serum creatinine and the BLB of some chelation group patients progressively rose several months following initial chelation therapy. Increasing BLB may emerge from

either bone lead stores[26] or environmental lead re-exposure from dusts, diet or water.[16] However, the increased serum creatinine levels again decreased after the BLB increase had again been reduced by repeated chelation therapy, suggesting that low-level environmental lead exposure is critical in accelerating progressive renal insufficiency. The findings of this study and previous investigations[15] suggest that repeated chelation therapy may effectively improve renal function and slow progressive renal insufficiency in most CKD patients with normal BLB (>20 μg and <600 μg). However, further studies are necessary to confirm such findings.

The mechanism by which lead chelation therapy improves renal function and retards the progression of renal insufficiency is unknown. Chronic low-level, not high-level, lead exposure may increase the level of reactive oxygen species and increase nitric oxide inactivation.[27,28] Lead-chelation therapy may reduce body lead and the levels of reactive oxygen species associated with nitric oxide inactivation in tissue, thus potentially improving renal function and slowing the progression in our patients.[27,28] However, the effects may also result from directly antioxidative effects[28] or removal of other ions[29] such as zinc, copper, iron of lead chelating agents by mechanisms other than reductions in BLB. Further studies are required.

Some limitations of this study were noted. Although the estimated GFR came from the serum creatinine, nitrogen, age, sex and daily protein intake, it indicated a strong association with the isotopic GFR ($r^2 = .91$).[20] Relative small sample size of the chelation group patients was noted, but it had adequate power estimated before the investigation. However, whether the findings can be applied to patients with multiple causes of CKD is unclear.

In conclusion, the findings of this study suggest that more CKD patients are adversely affected by environmental exposure to lead than was previously estimated. Fortunately, repeated lead chelation therapy may, in the long term, slow progressive CKD. However, further larger multicenter trials are needed to confirm the observation.

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