Supplemental Materials

Urinary Melamine Levels and Progression of Chronic Kidney Disease

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Supplemental method – urinary melamine measurement

Briefly, the elute of 1 ml urine sample collected in an Oasis MCX SPE cartridge (Waters, Malford, MA) was dried using nitrogen gas. The residues were reconstituted in 200 ml mobile phase and analyzed LC-MS/MS. The MDL in urine was 0.4 ng/ml. Urinary creatinine was analyzed by spectrophotometer (U-2000; Hitachi, Tokyo, Japan) set at a wavelength of 520 nm to measure the creatinine–picrate reaction.

Supplemental Tables

Supplemental Table 1. The clinical characteristics of CKD subjects of eGFR ≥ 30 ml/min/1.73m^2 with or without baseline urinary melamine levels.

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Supplemental Table 7. Comparison of demographic characteristics and laboratory data between patients with calcium urolithiasis and one cohort of occupational setting with melamine tableware manufacturing workers (melamine workers) and healthy controls

in our earlier study.

Supplemental Table 1. The clinical characteristics of CKD subjects of eGFR ≥ 30 ml/min/1.73m^2 with or without baseline urinary melamine levels.

	Entire Cohort	**Measured urinary melamine**		**Unmeasured urinary melamine**	
N	742	293		449	
Age (yrs)	62 ± 16	57 ± 14		65 ± 16	
Sex (male, %)	526 (71)	159 (54)		367 (82)	
eGFR (ml/min/1.73m^2)	56 ± 25	59 ± 29		53 ± 21	
	Entire Cohort	eGFR ≥ 60 ml/min/1.73m^2	eGFR ≥ 30 and < 60 ml/min/1.73m^2	eGFR ≥ 60 ml/min/1.73m^2	eGFR ≥ 30 and < 60 ml/min/1.73m^2
N	742	115	178	119	330
Sex (male, %)	526 (71)	66 (57)	93 (52)	83(70)	287(87)
Age (n, %)					
≤ 50 yrs	135(18)	36 (31)	43 (24)	34(29)	23(7)
>50-≤ 70 yrs	364(49)	65 (57)	100 (56)	62(52)	136(41)
>70 yrs	243(33)	14 (12)	35 (20)	23(19)	171(52)

Supplemental Table 2. The Spearman correlation between urinary corrected melamine levels and clinical data related to renal outcomes in study subjects.

	Baseline eGFR, ml/min/1.73m^2	**UPCR, g/g**	**Albumin, g/dl**	**Uric acid, mg/dl**	**Hemoglobin, g/dl**
Spearman's r	-0.32	0.15	-0.19	0.12	-0.26
P-value	<0.001	0.015	0.001	0.041	<0.001

Abbreviations: eGFR: estimated glomerular filtration rate; UPCR: urinary protein/creatinine ratio.

Supplemental Table 3. Distribution of cancer or other causes of death during follow up by tertiles of urinary corrected melamine levels

		Urinary corrected melamine levels		
	Entire cohort	**Tertile 1**	**Tertile 2**	**Tertile 3**
N	**293**	**98**	**97**	**98**
		N (%)		
Cancer or other causes of deaths	8(2.7)	1(1.0)	5(5.2)	2(2.0)

[a]Fisher's exact test.

Three tertiles of urinary corrected melamine levels (μg/mmol): 0.02-0.58, 0.59-1.46, and 1.47-12.7 with cut-offs of at 0.58 and 1.46 μg/mmol.

Supplemental Table 4. Relationship of urinary corrected melamine levels with different renal outcomes in subjects entering doubling of creatinine levels after 2 years of enrollment

Log_{10}-transformed urinary corrected melamine levels	**Adjusted HR[a] (95% CI)**
Doubling of serum creatinine levels	1.82(1.07-3.10)
	Adjusted OR[a] (95% CI)
eGFR decline > 3 ml/min/1.73 m^2 per year	2.00(1.11-3.62)
30% decrease of eGFR in the first two years	2.96(1.38-6.33)

Abbreviations: CI: confidence interval; eGFR: estimated glomerular filtration rate; HR: hazard ratio; OR: odds ratio.

[a]Adjusting for age, sex, body mass index, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker usage,
diabetes mellitus, hypertension, eGFR and UPCR (urinary protein/creatinine ratio) cut at 1 g/g.

Supplemental Table 5. Relationship of urinary uncorrected melamine levels and different renal outcomes adjusting for other covariates plus urinary creatinine levels

	Doubling of serum creatinine levels	**eGFR decline > 3 ml/min/1.73 m^2 per year**	**30% decline in eGFR in the first two years**
	HR (95%CI)	OR (95%CI)	OR (95%CI)
Crude analyses			
Log_{10}-transformed urinary melamine levels	2.00 (1.25-3.19)	1.93 (1.15-3.24)	2.41 (1.28-4.54)
By tertiles[a]			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.87 (1.06-3.32)	1.72 (0.94-3.14)	2.01 (0.94-4.28)
Tertile 3	2.03 (1.13-3.66)	1.78 (0.97-3.27)	2.65 (1.24-5.67)
Adjusted analyses[b]			
Log_{10}-transformed urinary melamine levels	1.81 (1.03-3.16)	2.03 (1.13-3.65)	2.49 (1.21-5.11)
By tertiles[a]			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.86 (1.01-3.43)	1.82 (0.94-3.53)	1.92 (0.85-4.32)
Tertile 3	1.89 (1.04-3.42)	1.81 (0.92-3.54)	2.52 (1.11-5.70)

Abbreviations: CI: confidence interval; eGFR: estimated glomerular filtration rate; HR: hazard ratio; OR: odds ratio

[a]Urinary uncorrected melamine level (ng/ml) cut at 4.36 and 10.60.

[b]Adjusting for age, sex, body mass index, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker usage, diabetes mellitus, hypertension, baseline estimated glomerular filtration rate, urinary protein/creatinine ratio cut at 1 g/g, and log-transformed urinary creatinine levels.

Three tertiles of urinary corrected melamine levels (μg/mmol): 0.02-0.58, 0.59-1.46, and 1.47-12.7 with cut-offs of at 0.58 and 1.46 μg/mmol.

Supplemental Table 6. Relationship of urinary corrected melamine levels with different renal outcomes by using the method of covariate-adjusted standardization

	Doubling of serum creatinine levels	**eGFR decline > 3 ml/min/1.73 m^2 per year**	**30% decline in eGFR in the first two years**
	HR (95%CI)	OR (95%CI)	OR (95%CI)
Crude analyses			
Log_{10}-transformed melamine levels	2.09 (1.33-3.29)	2.05 (1.25-3.39)	2.60 (1.41-4.80)
By tertiles[a]			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.68 (0.91-3.13)	1.72 (0.94-3.14)	1.26 (0.57-2.79)
Tertile 3	2.66 (1.50-4.72)	2.09 (1.15-3.79)	3.15 (1.53-6.45)
Adjusted analyses[b]			
Log_{10}-transformed melamine levels	1.77 (1.06-2.97)	2.07 (1.17-3.64)	2.64 (1.32-5.27)
By tertiles[a]			
Tertile 1	Reference	Reference	Reference
Tertile 2	1.58 (0.83-3.01)	1.75 (0.90-3.41)	1.10 (0.47-2.59)
Tertile 3	2.08 (1.13-3.84)	2.05 (1.04-4.03)	3.03 (1.37-6.74)

Abbreviations: CI: confidence interval; eGFR: estimated glomerular filtration rate; HR: hazard ratio; OR: odds ratio

[a]Predicted urinary melamine levels (μg/mmol creatinine) 0.58 and 1.53.

[b]Adjusting for age, sex, body mass index, angiotensin converting enzyme inhibitor/angiotensin II receptor blocker usage, diabetes mellitus, hypertension, baseline estimated glomerular filtration rate, and urinary protein/creatinine ratio cut at 1 g/g.

Three tertiles of urinary corrected melamine levels (μg/mmol): 0.02-0.58, 0.59-1.46, and 1.47-12.7 with cut-offs of at 0.58 and 1.46 μg/mmol.

Supplemental Table 7. Comparison of demographic characteristics and laboratory data between patients with calcium urolithiasis and one cohort of occupational setting with melamine tableware manufacturing workers (melamine workers) and healthy controls in our earlier study.

Variables	**Early CKD patients (This study)**	**Patients with calcium[a] urolithiasis**	**Healthy controls[b]**	**Melamine workers[b]**
N	**293**	**309**	**105**	**44**
		N (%) or Mean ± SD (median, IQR)		
Gender				
Male	159 (54)	226 (73)	97 (92)	21 (48)
Female	134 (46)	83 (27)	8 (8)	23 (52)
Age (years)	56.8 ± 14.0 (58.2, 49.7-66.7)	54.7 ± 12.8 (55.0, 47.0-63.0)	48.8 ± 9.0 (51.0, 48.0-55.0)	42.2 ± 9.0 (43.0, 34.0-50.0)
BMI (kg/m^2)	25.8 ± 4.1 (25.4, 23.1-28.2)	26.3 ± 4.1 (25.9, 23.8-28.4)	23.7 ± 2.7 (23.2, 22.0-25.5)	23.4 ± 4.7 (22.8, 20.7-25.0)
Urinary melamine levels				
Without correction (ng/ml)	11.63 ± 14.98 (7.05, 3.34-13.94)	12.30 ± 29.20 (4.86, 1.93-12.54)	8.1 ± 9.1 (4.3, 1.6-11.2)	456.0 ± 507.0 (269.6, 50.3-725.2)
With correction (μg/mmol Cr)	1.7 ± 2.1 (0.97, 0.43-2.08)	3.24 ± 6.66 (1.26, 0.48-3.29)	0.7 ± 0.9 (0.3, 0.1-1.0)	40.3 ± 45.6 (22.1, 4.3-58.3)

[a]Data from Liu CC, Wu CF, Chen BH, Huang SP, Goggins W, Lee HH, et al: Low exposure to melamine increases the risk of urolithiasis in adults *Kidney Int* 80: 746-752, 2011

[b]Wu CF, Peng CY, Liu CC, Lin WY, Pan CH, Cheng CM, et al: Ambient melamine exposure and urinary biomarkers of early renal injury. *J Am Soc Nephrol* 26: 2821-2829, 2015