# Follow-up results of children with melamine induced urolithiasis: a prospective observational cohort study

**Jian Gao, Hong Xu, Xin-Yu Kuang, Wen-Yan Huang, Nai-Qing Zhao, Jia Rao, Qiang-Ying Qian, Xian-Ying Cheng, Zhi-Min Feng, Jing Xu, Xin Zhang, Xiang Wang**

*Shanghai, China*

Original article

***Background:*** **Melamine-contaminated milk powder was the cause of the 2008 outbreak of urolithiasis in young children and infants in China, but the prognosis of these children remains unknown. We hypothesized that urolithiasis induced by melamine-contaminated milk powder may be associated with secondary renal injury.**

***Methods:*** **A total of 8335 children (≤6 years old) with a history of consuming melamine-contaminated milk powder were screened. Urine analysis and urinary system ultrasonography were performed. For children with urolithiasis, the basic information and the results of examination were recorded, and effective therapy was given. They were followed up for 6 months after the original diagnosis, and urinary microprotein profiles were measured.**

***Results:*** **Of the 8335 children, 105 (1.26%) were diagnosed with melamine-contaminated milk powder-associated urolithiasis. The size of the stone was correlated with the duration of exposure to melamine. Six months later, 69.8% (67) of the children with urolithiasis passed stones (follow-up rate: 91.4%). Of the 67 children, 28 passed stones within 2 months. The higher possibility of passing a stone was correlated with the smaller diameter of the stone ($P$<0.001). The detection rate of abnormal urinary microprotein excretion (microalbumin, immunoglobulin G, and N-acetyl-β-D-glucosidase) was 52.4% in children with persistent stones and 38.2% in those who passed their stones. The detection rate was lower in children who passed stones within 2 months (31.8%) than in those who passed stones in 2 to 6 months (50.0%). The levels of microalbumin/creatinine and immunoglobulin G/creatinine were significantly higher in children with persistent stones than in those who passed their stones.**

***Conclusions:*** **Early passage of a stone may reduce the renal injury induced by melamine-contaminated milk powder-associated urolithiasis.**

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***Key words:*** **children; follow up; melamine; renal injury; urolithiasis**

## Introduction

On September 11, 2008 the Ministry of Health of China confirmed that the outbreak of urolithiasis in young children and infants was caused by melamine-contaminated milk powder. Because of melamine's high nitrogen content (66.6%), it was added during the manufacturing process of milk powder to give falsely high protein content in food quality tests. More than 294 000 children were screened, of whom about 50 000 were hospitalized, and 6 died.[1] A recent epidemiological investigation[2] showed that urolithiasis is an independent factor for the development of chronic kidney disease (CKD). Moreover, some reports[3,4] showed that kidney stones in childhood could herald the deterioration of renal function and the development of hypertension. In animal experiments, melamine-induced acute kidney injury was mainly located in the renal interstitium,[5] whereas animals with urinary calculi but without acute renal injury could also develop CKD.[6] The 2007 melamine pet food incident in the US showed similar findings.[7] Since the outbreak of the melamine milk

**Author Affiliations:** Department of Nephrology, Children's Hospital of Fudan University, Shanghai 201102, China (Gao J, Xu H, Kuang XY, Huang WY, Rao J, Qian QY, Cheng XY, Feng ZM, Xu J, Zhang X, Wang X); Department of Biostatistics and Social Medicines, School of Public Health, Fudan University, Shanghai 201102, China (Zhao NQ)

**Corresponding Author:** Hong Xu, MD, PhD, Department of Nephrology, Children's Hospital of Fudan University, Shanghai 201102, China (Tel: 86-21-64931006; Fax: 86-21-64931901; Email: hxu@shmu.edu.cn)

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powder incident, relevant cases with kidney damage have been reported.[8,9] However the detailed baseline and prognosis of kidney damage remain unknown. We hypothesize an association between renal calculi induced by melamine-contaminated milk powder and short to medium term renal injury. This follow-up study aimed to investigate the outcomes of children with urolithiasis induced by melamine-contaminated milk powder and their secondary prognosis in addition to the effects on their kidneys.

## Methods

### Study subjects

From September 17 to October 15, 2008, a total of 8335 children (age less than or equal to 6 years) with a history of consuming melamine-contaminated milk powder were voluntarily screened at the Children's Hospital of Fudan University, Shanghai.

The study was approved by the Research Ethics Board, Children's Hospital of Fudan University, Shanghai, China. Parents or guardians provided informed consent for data acquisition.

### Screening

The basic information of screened children was collected, including sex, age, route of exposure, the melamine concentration consumed, the duration of exposure, urinalysis results, and urinary system ultrasonography. According to the results of the General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China, 22 brands of milk powder were tainted by melamine. Sanlu milk powder had the highest melamine concentration, ranging from 162 mg/kg to 2563 mg/kg. The melamine concentration of other brands of milk powder ranged from 0.09 mg/kg to 150 mg/kg in the screening.[10] Therefore, we defined Sanlu, Sanlu combined with other brand(s), and other brand(s) as having high, middle, and low concentrations of melamine, respectively.

Ultrasonography was performed by qualified ultrasonographic physicians using a 3.5-5 MHz probe (SEQUOIA 512, Siemens, Germany). The smallest diameter of detectable calculi was 1.0 mm. The diagnostic criteria of melamine-contaminated milk powder associated urolithiasis were: 1) the presence of a definite history of consuming melamine-contaminated milk powder before screening; 2) no urinary system disorders caused by other primary or secondary diseases; and 3) abnormal urinary system multi-sectional ultrasonography (clump of sharp hyper-echoic foci with or without acoustic shadow in the pelvis, calices of the kidney or ureter, punctiform sharp hyper-echoic foci with "comet-tail" artifacts, or acoustic shadow on gray-scale ultrasonography[11,12]).

The following information was obtained from patients with urolithiasis: the basic information of screened children, symptoms and findings on physical examination. Corresponding therapy was given immediately after diagnosis, while increasing fluid intake and alkalinizing the urine in all patients, and hospitalizing severe patients.

### Follow-up

The children with melamine-contaminated milk powder associated urolithiasis were followed up at 6 months after diagnosis. For those children who did not live in Shanghai or were unable to come back to our hospital for subsequent visits, the follow up was carried out by telephone conversation to record their symptoms, results of urinalysis and renal ultrasonography performed by local physicians. During the follow-up visit, renal and urinary tract ultrasonography was performed again.

A test of urinary microprotein profiles was commonly used as a sensitive marker for detecting glomerular and tubular injury. It can detect renal damage earlier and judge the source of proteinuria. When glomerular filtration membrane permeability increases or an electrostatic barrier is damaged, middle molecular and even giant molecular proteins, such as microalbumin (ALB) and immunoglobulin G (IgG), appear in urine. Hypomolecular proteins, such as N-acetyl-β-D-glucosidase (NAG), have concentrations high enough in renal tubular epithelial cells that their appearance in the urine indicates that the renal tubule has been damaged.[13,14] Testing of these proteins is more precise and sensitive than routine urine analysis and can detect kidney damage earlier, allowing for close observation and early interventional treatment. Therefore, we used it to assess renal damage in children with urolithiasis induced by melamine-contaminated milk powder. Urinary microprotein profiles were measured in our hospital, including microalbumin/creatinine (ALB/CR, reference range: 0 mg/g to 26.5 mg/g[15]), immunoglobulin G/creatinine (IgG/CR, reference range: 0 mg/g to 14 mg/g[16]), and N-acetyl-β-D-glucosidase/creatinine [NAG/CR, reference range: 0.3 to 1.2 U/mmol (Wako Co. provided the detection range)]. When examining the results of the urinary microprotein profiles, we classified an elevation of one or both of ALB/CR and IgG/CR levels as evidence of glomerular injury and the elevation of NAG/CR level as evidence of renal tubular injury.

### Statistical analysis

Quantitative data about glomerular and tubular injury

were transformed into qualitative data (normal and abnormal). The other variables, such as age, the duration of exposure to melamine, the concentration of melamine consumed and the size of calculi, were also transformed into categorical data. The distribution of each group was expressed as frequencies and percentages. A Chi-square test or Fisher's exact test was used to analyze the categorical data as appropriate. Quantitative data were expressed as mean ± SD. Student's *t* test or one-way ANOVA was used to compare quantitative data as appropriate. In all patients ($n$=105), Spearman's rank-order correlation coefficient analysis and the Cochran-Mantel-Haenszel test were used to evaluate the association between the diameter of calculi at initial presentation and the duration of exposure to melamine. An ordinal regression model was used to analyze the influencing factors of the size of calculi [$n$=105; odds ratio (OR) = $e^{Estimate}$, 95% confidence interval (CI) = $e^{95\%\ confidence\ interval\ of\ estimate}$], and sex, age, melamine concentration, and duration of exposure were included. A logistic regression model was used to analyze the influencing factors of glomerular and/or tubular injury and of stones status (passed or persistent, $n$=90) after 6 months, and a linear regression model was used to analyze the influencing factors of ALB/CR, IgG/CR and NAG/CR levels separately ($n$=76), in which sex, age, duration of exposure, size of calculi at initial presentation, and stones status during the follow-up were included. A two-tailed $P$ value less than 0.05 was considered statistically significant except for partitions of the Chi-square test. Statistical analyses were performed with SPSS version 11.5 and SAS version 9.0.

## Results

### Screening

The basic information collected from the 8335 children is summarized in Table 1.

Urinary tract calculi were detected in 105 (1.26%) of the 8335 children, of whom 77 were asymptomatic. Twenty-seven children presented with symptoms such as vomiting, unexplained crying, fever, anepithymia, and urinary tract infection. Only one child (1.0%) presented with acute renal failure (male, 8 months old, with a serum creatinine level of 712 mmol/L at initial presentation; he recovered quickly after ureteric retrograde catheterization). We did not find other causes of urolithiasis by clinical or laboratory examinations, such as hypercalcinuria, cystine metabolic abnormality or congenital abnormalities of the urinary tract.

In children with urolithiasis with a male/female ratio of 1:0.6, the detection rates of urolithiasis were significantly different between the male and female children (1.96% and 0.76%, respectively; $\chi^2$=22.710, $P$<0.001). They ranged from 1 month to 6 years old. The detection rates of urolithiasis were significantly different in different age groups ($\chi^2$=11.021, $P$=0.004), in which infants were more prone than 1-6 years old children to develop melamine-contaminated milk powder associated urolithiasis. The detection rates were also significantly different between the groups with different levels of melamine exposure ($\chi^2$=74.895, $P$<0.001), indicating that the children who were exposed for a longer period were more likely to develop urolithiasis (Table 1).

In 105 children with urolithiasis, the diameter of stones ranged from 1.1 mm to 19.3 mm, and the duration of exposure ranged from 1 month to 36 months. Among the groups exposed to melamine, the stone diameters were significantly different (Fisher, $P$=0.007). There was a positive, linear relationship between the duration of exposure to melamine and the diameters of stones (Spearman's rank-order correlation coefficient, $r$=0.262; $P$<0.010; and the Cochran-Mantel-Haenszel test, $P$=0.006). Ordinal regression analysis showed that patients who exposed to melamine for more than 12 months tended to form larger stones than those who exposed to melamine for less than or equal to 6 months ($P$=0.008). These results suggest that the longer duration of exposure to melamine may lead to the formation of bigger stones (Table 2).

### Follow-up

Six months later, 96 of the 105 children with urolithiasis were followed up (follow-up rate: 91.4%, 62 boys and 34 girls). Eighty-two children were followed up in our hospital, and 14 children were followed up by telephone. In 9 children who were lost to follow-up, 6 children provided wrong address and telephone number,

**Table 1.** Information about children screened and children with urolithiasis

| Characteristics | Children screened, *n* (%) | Children with urolithiasis, *n* (%) | *P* |
|---|---|---|---|
| Gender | | | |
| Male | 3473 (41.7) | 68 (1.96) | <0.001 |
| Female | 4862 (58.3) | 37 (0.76) | |
| Age (mon) | | | |
| 0 to ≤12 | 2488 (29.8) | 47 (1.89) | |
| >12 to ≤36 | 4765 (57.2) | 48 (1.01) | 0.004 |
| >36 to ≤72 | 1082 (13.0) | 10 (0.92) | |
| Concentration of consumed melamine | | | |
| High | 2284 (27.4) | 64 (2.80) | |
| Middle | 617 (7.4) | 15 (2.43) | <0.001 |
| Low | 5443 (65.3) | 26 (0.48) | |

*P* values were calculated using the Chi-square test.

and 3 were unwilling to cooperate. One child suffered from macroscopic hematuria and the rest did not show any specific symptoms related to urinary tract calculi, such as oliguria, anuria, renal colic, and grit in urine.

In the 96 children who underwent ultrasonography, 67 (69.8%) had passed their stones. Twenty-eight children passed their stone within 2 months after initial visit to the hospital. Logistic regression analysis showed that children with a small stone ($P$<0.001) were more likely to pass their stones (Table 3). In 29 (30.2%) children with persistent stones after 6 months, calculi diameters of ≤5 mm, >5 mm to ≤10 mm, and >10 mm were seen in 17, 7, and 5 children respectively. Their stones' sizes did not increase after 6 months. In 11

**Table 2.** Multivariate analysis using ordinal regression for comparison of data about children with urolithiasis at initial presentation and different calculi diameters

| Characteristics | Diameter of calculi (mm)*, *n* (%) | | | Estimate† | Odds ratio (95% CI) | *P* |
|---|---|---|---|---|---|---|
| | ≤5 | >5 to ≤10 | >10 | | | |
| Gender | | | | | | |
| Male | 45 (66.2) | 17 (25.0) | 6 (8.8) | -0.310 | 0.734 (0.312-1.728) | 0.479 |
| Female | 22 (59.5) | 7 (18.9) | 8 (21.6) | 0 | 1 | |
| Age (mon) | | | | | | |
| 0 to ≤12 | 30 (63.8) | 11 (23.4) | 6 (12.8) | 1.479 | 4.389 (0.702-27.41) | 0.114 |
| >12 to ≤36 | 29 (60.4) | 12 (25.0) | 7 (14.6) | 1.380 | 3.975 (0.719-21.98) | 0.114 |
| >36 to ≤72 | 8 (80.0) | 1 (10.0) | 1 (10.0) | 0 | 1 | |
| Concentration of consumed melamine | | | | | | |
| High | 37 (57.8) | 16 (25.0) | 11 (17.2) | 1.061 | 2.889 (0.882-9.469) | 0.080 |
| Middle | 9 (60.0) | 4 (26.7) | 2 (13.3) | 1.400 | 4.055 (0.871-18.88) | 0.074 |
| Low | 21 (80.8) | 4 (15.4) | 1 (3.8) | 0 | 1 | |
| Duration of exposure (mon) | | | | | | |
| 0 to ≤6 | 29 (82.9) | 6 (17.1) | 0 (0.0) | -1.912 | 0.148 (0.036-0.602) | 0.008 |
| >6 to ≤12 | 25 (53.2) | 13 (27.7) | 9 (19.1) | -0.383 | 0.682 (0.211-2.203) | 0.522 |
| >12 | 13 (56.6) | 5 (21.7) | 5 (21.7) | 0 | 1 | |

*: Determined using ultrasonography; In the presence of multiple stones, the largest diameter was used. †: The estimated value of β.

**Table 3.** Multivariate analysis by logistic regression for comparison of data about children with passed and persistent stones after 6 months*

| Characteristics | | Persistent stones, *n* (%) | Passing stones, *n* (%) | Odds ratio (95% CI) | *P* |
|---|---|---|---|---|---|
| Sex | | | | | 0.704 |
| Male | | 16 (25.8) | 46 (74.2) | 1.271 (0.368-4.394) | 0.704 |
| Female | | 13 (38.2) | 21 (61.8) | 1 | |
| Age (mon) | | | | | 0.106 |
| 0 to ≤12 | | 7 (17.1) | 34 (82.9) | 0.115 (0.015-0.862) | 0.035 |
| >12 to ≤36 | | 18 (39.1) | 28 (60.9) | 0.287 (0.051-1.618) | 0.157 |
| >36 to ≤72 | | 4 (44.4) | 5 (55.6) | 1 | |
| Concentration of consumed melamine | | | | | 0.312 |
| High | | 21 (36.2) | 37 (63.8) | 2.840 (0.639-12.635) | 0.170 |
| Middle | | 4 (30.8) | 9 (69.2) | 1.135 (0.163-7.911) | 0.898 |
| Low | | 4 (16.0) | 21 (84.0) | 1 | |
| Duration of exposure (mon) | | | | | 0.216 |
| 0 to ≤6 | | 7 (20.6) | 27 (79.4) | 0.829 (0.174-3.955) | 0.814 |
| >6 to ≤12 | | 11 (27.5) | 29 (72.5) | 0.281 (0.060-1.317) | 0.107 |
| >12 | | 11 (50.0) | 11 (50.0) | 1 | |
| Diameter of calculi (mm) | | | | | <0.001 |
| >10 | | 8 (80.0) | 2 (20.0) | 29.982 (3.834-234.485) | 0.001 |
| >5 to ≤10 | | 11 (50.0) | 11 (50.0) | 9.181 (2.514-33.506) | 0.001 |
| ≤5 | | 10 (15.6) | 54 (84.4) | 1 | |
| Urinalysis (*n*=85)† | | | | | *P*‡ |
| Proteinuria | - | 21 (26.6) | 58 (73.4) | | 0.009 |
| | +§ | 5 (83.3) | 1 (16.7) | | |
| Microscopic hematuria | - | 18 (25.0) | 54 (75.0) | | 0.018 |
| | + | 8 (61.5) | 5 (38.5) | | |
| Leukocyturia | - | 20 (28.2) | 51 (71.8) | | 0.344 |
| | + | 6 (42.9) | 8 (57.1) | | |

*: The enter method was used; †: Of 96 follow-up patients, 85 underwent urinalysis; ‡: The urinalysis results between patients with passed and persistent stones were compared. Fisher's exact test and the Chi-square test (microscopic hematuria) were used to calculate *P* values; §: Patients with proteinuria comprised those who had microscopic hematuria but urinary tract infection.

children with >10 mm stones at initial visit, 2 children passed their stones spontaneously within 6 months, 4 showed decreased stones, and 5 showed no change. The largest stone had a diameter of 13.1 mm.

We obtained 85 urine specimens from the 96 children at follow-up. The incidences of proteinuria, microscopic hematuria and leukocyturia were 7.1%, 15.3% and 16.5%, respectively (Table 3). Urinary microprotein profiles were measured in 76 of the 85 urine specimens of the children. The total rate of abnormality was 42.1% (32 children). In the 32 children, 52.4% had persistent stones, and 38.2% passed their stones. The detection rate of abnormality was lower in the children who passed stones within 2 months (31.8%) than in those who passed stones in 2-6 months (50.0%), suggesting that the earlier passage of stones may be associated with less renal injury. All children with >10 mm stones at initial visit had glomerular and/or tubular injury regardless of whether they had stones at follow-up.

The detection rate of glomerular injury was 32.9% (25 children: 12 had elevated ALB/CR alone, 3 had single elevated IgG/CR alone, and 10 had elevated ALB/CR and IgG/CR). Univariate analysis showed significant difference in the detection rates of glomerular injury between the different stone status groups ($\chi^2$=4.992, $P$=0.025), between the different calculi size groups (Fisher, $P$=0.001), and between the different melamine concentration consumed groups ($\chi^2$=8.894, $P$=0.012). A logistic regression analysis showed that a large stone at initial visit ($P$=0.017) and a high melamine concentration consumed ($P$=0.032) were risk factors for glomerular injury. The detection rate of tubular injury was 21.1% (16 children). Univariate analysis showed significant differences between the different age groups (Fisher, $P$=0.038) and between the different calculi size groups (Fisher, $P$=0.048). A logistic regression analysis showed that a stone >10 mm was a risk factor for tubular injury ($P$=0.010). The detection rate of both glomerular and tubular injury was 10.5% (8 children). Univariate analysis showed significant difference between the different stone status groups (Fisher, $P$=0.028) and between the different calculi size groups (Fisher, $P$=0.033). A logistic regression analysis showed that persistent stone after 6 months was a risk factor for both glomerular and tubular injury ($P$=0.024) (Table 4).

Urinary microprotein profiles were also quantitatively analyzed. Linear regression showed that the high level of ALB/CR was related to the presence of stones after 6 months ($B$=111.415, 95% CI: 41.282–181.549, $P$=0.002) and a long exposure to melamine ($B$=45.110, 95% CI: 0.790–89.431, $P$=0.046); the level of IgG/CR was related to the presence of persistent stones after 6 months ($B$=14.369, 95% CI: 4.384–24.355, $P$=0.006); and the level of NAG/CR was related to the large diameter of a stone ($B$=0.162, 95% CI: 0.034–0.290, $P$=0.014) and young age ($B$=-0.194, 95% CI: -0.321–0.068, $P$<0.001). These results indicate that the children with persistent stones and/or a long exposure to melamine may have a high level of ALB/CR and/or IgG/CR, and young children and/or those with large stones may have a high level of NAG/CR after 6 months.

**Table 4.** Logistic regression analysis of risk factors for glomerular and/or tubular injury in children with urolithiasis*

| Injured sites | Risk factors | Odds ratio (95% CI) | $P$ |
|---|---|---|---|
| Renal glomerulus | Diameter of calculi | | 0.028 |
| | >10 mm | 17.433 (1.685-183.460) | 0.017 |
| | >5 mm to ≤10 mm | 2.813 (0.826-9.573) | 0.098 |
| | ≤5 mm | 1 | |
| | Concentration of consumed melamine | | 0.033 |
| | High | 5.876 (10167-29.590) | 0.032 |
| | Middle | 1.144 (0.118-11.125) | 0.908 |
| | Low | 1 | |
| Renal tubular | Diameter of calculi | | 0.033 |
| | >10 mm | 18.604 (1.991-173.797) | 0.010 |
| | >5 mm to ≤10 mm | 0.900 (0.191-4.236) | 0.893 |
| | ≤5 mm | 1 | |
| Glomerular and tubular | Stones status | | 0.024 |
| | Persistent | 8.949 (1.328-60.294) | 0.024 |
| | Passing | 1 | |

*: The table only shows the significant factors of all results for three dependent variables. All independent variables included sex, age, duration of exposure, size of calculi at initial presentation, and stone status during follow-up.

## Discussion

In the present study, more than two-thirds of children with melamine-contaminated milk powder associated urolithiasis passed their stones spontaneously in 6 months. Over 40% of the children including those who had passed their stones suffered from renal injury after 6 months. The finding suggests that passing a stone earlier may lead to less renal injury and that the factors causing renal injury are the size of stone, age, melamine concentration and stone status.

The exact incidence of pediatric urolithiasis in China is not clear. However, China by no means is a prevalent region for pediatric urolithiasis in the world. For many decades, metabolic abnormalities were considered the main cause of pediatric urolithiasis (40%).[17] In the present study, 105 of all children of 6 years old or younger who were screened at our hospital were identified as having melamine-contaminated

milk powder associated urolithiasis. Because of lack of techniques for detecting melamine in the urine or blood in China, we do not have detailed data about melamine content in the body. We used the diagnostic criteria provided by the Ministry of Health of China.[18] It is common for preschoolers to consume milk powder in China. We also found urolithiasis in some older children (9.0%). Hence we increased the enrollment age range to 6 years old. The detection rate of urolithiasis in our hospital was 1.26% in all children of 6 years old or younger who consumed melamine-contaminated milk powder. According to recent reports, the detection rate of urolithiasis is 8.5% in Beijing (50 among 589 children screened, age 0 to 36 months),[8] 0.4% in Hong Kong (8 among 2140 children screened, age 1 month to 12 years),[11] and 1.1% in Taiwan province (12 among 1129 children screened, mean age: about 4 years).[19] Different detection rates may be related to various factors such as different regions, different concentrations of melamine consumed, patient age, and the number of screened children.

The impact of urinary calculi on the kidneys is thought to be physical injury.[5,7] But melamine-induced stones may cause different problems. Guan et al[8] reported that 9.8% of 44 children with nephrolithiasis, 13.6% of 88 children with suspected stones, and 5.6% of 269 children without urinary stones (control group) had abnormal glomerular function. Lam et al[9] reported that urinalyses of 15 cases of urolithiasis caused by melamine revealed proteinuria in 3 cases, abnormal urinary ALB in 2, and abnormal urinary β2-microglobulin in 2. The results suggest that melamine-contaminated milk powder-associated urolithiasis may lead to glomerular and tubular injury. However, the number of cases screened has been relatively small in recent reports.

In the present study 105 of the 8335 children who had been initially screened were followed up. We analyzed the outcome and effects of contaminated-milk powder associated urolithiasis on renal injury.

After entering the human body, 90% of melamine is excreted through the kidneys within 24 hours.[20] During the digestive process, melamine can be combined with uric acid to form a large insoluble compound, the melamine-uric acid crystal.[21] The main organ influenced by melamine is the urinary system. Melamine can lead to the formation of stones in the bladder, ureter, and kidneys.[22,23] In our follow-up of children with urolithiasis, single and ≤5 mm stones were frequently seen. The detection rate of urolithiasis was higher in boys than in girls. Young infants are more susceptible to the disease because they depend solely on milk and are increasingly exposed to melamine. Moreover they have a short duration between meals (usually 2 to 3 hours), which leads to high accumulation of melamine in blood and urine, resulting in crystallization of stones. The concentration of melamine in milk powder may be one of the important factors causing urolithiasis. We suggest that the duration of exposure to melamine affects not only the formation[24] but also the size of stones. This can explain why large stones are found in children consuming melamine at a low concentration.

Our findings suggest that the size of a stone is one of the important factors for passing urinary calculi. The duration of contaminated milk powder consumption is not a direct influencing factor for passing stones, but it is related to the size of stone and could indirectly affect passing stones. Interestingly, we found in the present study that the proportion of stones passed spontaneously after 6 months in infants was higher than that in young children although the detection rate of urolithiasis in the former was higher than in the latter. These differences indicate that the infants with urolithiasis may recover more quickly.

More than 95% of the melamine absorbed can be spontaneously excreted. However, studies demonstrated that cyanuric acid (congener of melamine) can accumulate in the tissue.[25,26] Sub-chronic toxicity tests revealed that animals fed with melamine for a long period could develop renal injury.[6,27,28] However, it remains unclear whether chronic ingestion of melamine will affect human kidney function. A recent epidemiologic investigation[2] showed that urolithiasis is an independent factor inducing the development of chronic kidney disease. Therefore, melamine-associated stones may lead to long-term renal injury.

In our study, urinary microprotein profiles showed a high detection rate of glomerular and/or tubular injury in children with melamine-associated stones after 6 months. In these children, 22 had passed stones, but renal injury still existed. We identified the size of the stones, the concentration of melamine consumed and stone status after 6 months as the main factors causing renal injury. Interestingly, there was a sex difference in the incidence of stones, i.e., the higher incidences of stones and renal tubular injury in boys in contrast to the higher incidences of large stones, proteinuria, and glomerular injury in girls. Although we did not observe the baseline level of urinary microprotein profiles in patients with urolithiasis at initial presentation, the follow-up results indicate that long-term monitoring is needed for the renal function of the patients.

The results of our study also raise 3 questions. First, while more than two thirds of children pass stones within 6 months, how long will the stones persist? Second, children with melamine-associated urolithiasis have different degrees of renal injury, but would this

injury last for a long time? Third, is renal injury caused by urolithiasis itself or the chronic renal toxicity of melamine?

We found that the rate of passing stone was higher in patients with <5 mm stones (84.4%) than in those with 5 mm to 10 mm stones (50%). Of 14 patients with >10 mm stones at initial presentation, only 2 patients passed their stones spontaneously. The other 12 patients did not have macrohematuria, oliguria, anuria, or urinary obstruction, but the proteinuria and urinary microprotein profiles were abnormal. These patients should be monitored closely so as to treat them timely before the disease deteriorates.

We determined the abnormal detection rate of urinary microprotein profiles in those patients who passed their stones. In the children who passed stones within 2 months there was a lower abnormal detection rate of urinary microprotein profiles than in those who passed stones beyond 2 months. This difference suggests that passing stones earlier would reduce the incidence of renal injury.

The present study has some limitations. First, we cannot control bias in patient selection caused by the geographic location of the children screened and repeated visits to our hospital after diagnosis at other hospitals. Second, we cannot exclude confounding bias caused by non-melamine-related renal stones. Third, we did not have the data from the urinary microprotein profiles of patients at the initial presentation.

In conclusion, screening and follow-up for 6 months can detect persistent stones in some patients. Early passing of a stone may lead to less renal injury. Renal injuries in children with melamine-associated stones need to be closely monitored for a long period, and injuries of other organs will be observed in our future study.

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