Objectives: Melamine was known as a new risk for kidney stone due to recent incidences of milk powder contamination in China. Here, we performed a retrospective study to investigate whether age, gender, and urinary pH affect melamine-associated kidney stone risk.

Materials and Methods: A retrospective review was performed of 217 children aged less than 3 years old. All children had a history of being fed with Sanlu milk powder contaminated by melamine, and underwent a clinical screening on kidney stone in Shenyang from November 2008 to February 2009. A comparison with the Chi-square was conducted between 83 cases and 125 normal subjects. The difference between children's gender, age, and urinary pH was evaluated.

Results: A total of 208 subjects, 136 boys and 72 girls, were included in the study. Significant association was observed between melamine-associated kidney stone risk and gender (odds ratio [OR], 2.03; 95% confidence interval [CI], 1.11-3.74; \( P = 0.02 \)) and urinary pH (OR, 1.78; 95% CI, 1.01-3.11; \( P = 0.04 \)), respectively. Male children were at about twofold increased melamine-associated kidney stone risk compared with female children. Acidic urine showed about 1.78-fold increased melamine-associated kidney stone risk compared with normal urine.

Conclusions: Our investigation results showed an association of gender and urinary pH with melamine-associated kidney stone formation risk.

Key Words: Kidney stone, melamine, urinary pH
raise the apparent protein content of these products. About 3,000,000 children have been affected by milk powder contaminated with melamine in China.1-2 Several investigations provided with substantial information about the relationship between exposure to melamine-containing milk powder and nephrolithiasis in infants and children.3-5. However, these observational data reveal an association between intake dose of melamine and prevalence of kidney stones. It is still unclear whether some clinical factors associated with common calcium oxalate stone, such as urinary pH, gender, and age may affect melamine-associated kidney stone formation risk. Here, we investigated the relationship among melamine-associated kidney stone risk with age, gender, and urinary pH.

MATERIALS AND METHODS

Subjects
A retrospective review was performed of 217 children aged less than 3 years (mean age, 1.8±0.8 years). All children had a history of feeding Sanlu milk powder contaminated with melamine, and underwent a clinical screening for kidney stone at the Fourth and Eighth People’s Hospital in Shenyang from November 2008 to February 2009. Most of them attended our clinic as outpatients. Standard urine tests and renal ultrasound examinations were carried out in all children. The presence of a kidney stone was defined as evidence on both (1) renal ultrasound with hyperechogenic spots in kidney or ureters, and (2) microscopic hematuria with five or more red blood cells per high-power field in a centrifuged urine sample. Urinary pH of less than 6.5 was defined as acidic urine. Considering the possibility of hemorrhage disease or glomerulnephritis, we excluded nine cases with hematuria. A comparison was made between kidney stone cases and normal children. The difference of children gender, age, and urinary pH was evaluated. Considering that 1-year-old children will add some solid food intake except for milk powder, we classified children into two groups, 0- to 1-year-old and 1- to 3-year-old. All study subjects came from the same area and with similar socioeconomic backgrounds. The Institutional Review Board approved the study protocol.

Statistics
An association between clinical feature and risk of melamine-associated kidney stone was analysed with the Chi-square test. Statistical analysis was performed using the Statistical Package for Social Sciences (version 10.0; SPSS Inc., Chicago, IL, USA). The odds ratios (OR) and 95% confidence interval (CI) were calculated to determine the risk of melamine-associated kidney stone. \( P \leq 0.05 \) was considered as significant.

RESULTS

All children had a history of feeding Sanlu milk powder during past 3 months. A total of 208 subjects — 136 boys and 72 girls — were included in the present study. Of 208 children, 83 cases were diagnosed with melamine-associated kidney stone (39.9%). Of these, 125 had no kidney stone. Most children with stones did not have oliguria, crying on urination, oedema, or passing of stones.

Gender and melamine-associated kidney stone risk
The cases included 62 boys and 21 girls. The boys to girls ratio was 3.0:1.0. Normal children included 74 boys and 51 girls. We tested the association of gender with melamine-associated kidney stone risk between 83 cases and 125 normal subjects [Table 1]. Gender was significantly associated with melamine-associated kidney stone risk (OR, 2.03; 95% CI, 1.11-3.74, \( P=0.02 \)). Male children were at 2.03-fold increased melamine-associated kidney stone risk compared with female children.

Age and melamine-associated kidney stone risk
Subjects were grouped into an infant group with less than one year olds and a group with one to three year olds. The correlation between age and melamine-associated kidney stone risk was evaluated [Table 1]. There was no significant association between different age stages and melamine-associated kidney stone risk (OR, 0.95; 95% CI, 0.35-2.57; \( P=0.93 \)).

Urinary pH and melamine-associated kidney stone risk
A total of 105 children had acidic urine. The correlation between urinary pH and melamine-associated kidney stone risk was evaluated [Table 1]. Urinary pH was significantly associated with melamine-associated kidney stone risk (OR, 1.78; 95% CI, 1.01-3.11; \( P=0.04 \)). Acidic urine was at about 1.78-fold increased melamine-associated kidney stone risk compared with normal urine.

DISCUSSION

As a new type of kidney stone, the features of melamine-associated kidney stone are rarely known. In the present study,

| Table 1: Association analysis of gender, age, and urine pH between melamine kidney stone cases and normal subjects |
| --- | --- | --- | --- |
| Sex | Cases Normal subjects | OR | 95%CI | Chi square |
| Male | 62 | 74 | 2.0347 | 1.106-3.745 | 5.294 | 0.0214* |
| Female | 21 | 51 | 1.0000 | 1.0000-1.0000 | 0.0000 | 1.0000 |
| Age | 0.9545 | 0.354-2.572 | 0.008 | 0.9266 |
| ≤1y | 7 | 11 | 0.9545 | 0.354-2.572 | 0.008 | 0.9266 |
| 1-3y | 76 | 114 | 1.0000 | 1.0000-1.0000 | 0.0000 | 1.0000 |
| Urine PH | 1.7757 | 1.013-3.115 | 4.044 | 0.0443* |
| <6.5 | 49 | 56 | 1.0000 | 1.0000-1.0000 | 0.0000 | 1.0000 |
| ≥6.5 | 34 | 69 | 1.0000 | 1.0000-1.0000 | 0.0000 | 1.0000 |

CI - Confidence Interval; OR - Odds Ratio, * \( P<0.05 \).
melamine-associated kidney stone was observed to have a significant association with urinary pH and gender.

Urinary pH has been reported to be associated with common kidney stone. The present findings support an association between melamine-associated stone formation and urinary pH. We considered two possible explanations for this. First, urinary pH may affect urinary supersaturation with regard to melamine. Supersaturation is the driving power for crystal formation. Urinary pH is the main determinant of supersaturation with respect to certain components. Increasing the urinary pH can raise the upper limit of metastability and prevent crystal formation. Previous toxicology studies reported that melamine does not undergo significant metabolism and can be primarily cleared by renal filtration due to its properties of being a small polar molecule. Increase in melamine component in urine may lead to increased supersaturation with respect to melamine. Lower urinary pH will be associated with a higher risk of melamine-associated kidney stone formation. Second, urinary pH may affect the formation of insoluble complexes between cyanuric acid and melamine. Cyanuric acid, structurally related to melamine, is a by-product of melamine metabolism. Melamine and cyanuric acid can rapidly combine into a lattice structure via hydrogen bonds at pH 5.8. It is easier to form melamine-cyanurate crystals in acid urine.

The gender epidemiology of kidney stone disease has been conventionally reported to have a male-to-female ratio of 3:1. However, the sex-specific prevalence in the paediatric population has not yet been well defined. It is more limited knowledge in melamine-associated kidney stone. In the present study, we showed a sexual association with melamine-associated kidney stone, and reported a male-to-female ratio of 2.4:1 in melamine-associated kidney stone in Shenyang of China, which is similar to ratio of common calcium oxalate kidney stone. However, our data concerning gender are not in agreement with previous investigation by Guan et al. in Beijing. A male-to-female ratio can be calculated to be about 3:2 base on their data. In others studies, there was not detail description between gender and melamine-associated kidney stone. The different incidences may have resulted from uric acid levels between male and female children was 5.82 mg/dl and 4.33 mg/dl, respectively. The different uric acid levels between male and female children may be due to sex steroids, which play a significant role in uric acid regulation in biological fluids. In addition, oestrogen may result in lower urinary calcium excretion and calcium oxalate saturation. These factors may lead to a lower incidence of melamine-associated kidney stone for female than male children.

In the present study, certain limitations must be considered while interpreting the study findings. First, geographical and environmental factors, such as climate and economy developing level may lead to a diversity investigation results between different region. The subjects in the present study were recruited from Northern China, which has a colder climate and correspondingly underdeveloped economy than in Southern China. These factors may affect prevalence of kidney stone. Second, individual risk factors, such as weight index and exposure dose of melamine, and stone analysis have not been established in the present study due to the lack of relevant clinical information in outpatient screening. These factors may affect evaluated results to a certain extent.

In summary, our investigation results showed an association of melamine-associated kidney stone formation risk with gender and urinary pH. However, the mechanism of how urinary pH and sex hormone are involved in melamine-associated kidney stone formation remains unclear. Melamine has been widely appended in domestic animals feed and milk powder in recent years, and also been found to exist in some daily plastic necessities. It is not known whether melamine may lead to accumulative effect in human body. Therefore, it is essential to further investigate this in future.

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