Melamine-contaminated milk products induced urinary tract calculi in children

Li Zhang, Ling-Ling Wu, Ya-Ping Wang, Ai-Min Liu, Chao-Chun Zou, Zheng-Yan Zhao

Hangzhou, China

Background: **Melamine is an industrial chemical used primarily as plastics stabilizer and fire retardant. On September 11, 2008, melamine-contaminated milk products were reported to be responsible for urinary tract calculi in infants and children in China. This study aimed to investigate the prevalence, lesions, risk factors, clinical features, and management of children fed with the melamine-contaminated milk products.**

Methods: **A total of 15 577 infants and children fed with the milk products were screened at our hospital. Ultrasonography was performed in all the infants and children. For those found with urinary tract calculi on ultrasonography, urnalysis was done. Among them, 846 with detailed data screened from September 17 to 25 were enrolled for further analysis in this study. They were divided into calculus group (326 children) and non-calculus group (520 children) according to the results of ultrasonography. They included 429 boys and 417 girls, aged from 1 month to 5 years (median, 18 months). Their clinical and laboratory data, ultrasonograms, and treatment results were analyzed.**

Results: **Of the 15 577 children screened, 562 (3.61%) had urinary tract calculi. The rate was closely related to the melamine concentration in patients fed with formula. In 846 children with detailed data enrolled in this analysis, weight and head circumference *Z* scores in the calculus group were lower than those in the non-calculus group (*P*=0.048, *P*=0.046). Long duration of formula feeding, high melamine contained formula, and minimal water intake were the risk factors for calculi (*P*<0.05, respectively). Of 326 children with calculi, 281 had small calculi less than 0.5 cm in diameter, 227 had multiple calculi, and 34 had urinary tract distention. Moreover, diffuse renal lesions, renal failure and ascites were noted in 4, 3 and 2 patients, respectively. After 1-month treatment with sodium bicarbonate and Chinese traditional medicine, calculi disappeared in 49 of 54 outpatients. In 41 inpatients, 5 had calculi removed operatively and 36 had calculi minimized.**

Conclusions: **Melamine-contaminated milk products induced urinary tract calculi, which have a good response to conservative therapy. Long-term follow-up of infants and children fed with melamine-contaminated milk products is required, and food safety should be supervised increasingly for the health of children.**

World J Pediatr 2009;5(1):31-35

Key words: **food contamination;**
infant;
infant formula;
melamine;
urinary caculi

Introduction

Melamine, also called cyanuramide or triamino-triazine, is a nitrogenous chemical substance. Structurally it consists of 2, 4, 6-triamino-1, 3, 5-triazine ($C_3H_6N_6$), produced from several cyanogen compounds. As a white crystalline substance, one of the heterocyclic organic compounds, it is used principally as a starting material for the manufacture of synthetic resins. Usually formulated with fillers and pigments, melamine is molded into dishes, containers, utensils, handles, and the like or used as a laminating agent or coating material for wood, paper, and textiles.

Melamine can increase the nitrogen concentration in food. Pet's food contaminated by melamine once resulted in insoluble crystals that obstruct and damage renal tubules and cause renal failure.[1,2] But no data are available on food contaminated by melamine in human. Some doctors and parents have doubted about the relationship between calculi of the urinary system and consumption of Sanlu milk formula since

Author Affiliations: Department of Children's Health Care (Zhang L, Wu LL, Zou CC, Zhao ZY) and Department of Nephrology (Wang YP, Liu AM), The Children's Hospital of Zhejiang University School of Medicine and Zhejiang Key Laboratory for Diagnosis and Therapy of Neonatal Diseases, Hangzhou 310003, China

Corresponding Author: Chao-Chun Zou, The Children's Hospital of Zhejiang University School of Medicine, 57 Zhugan Xiang, Hangzhou 310003, China (Tel: +86-15067123060; Fax: +86-571-87033296; Email: zou108cc@yahoo.com)

doi:10.1007/s12519-009-0005-6

December 2007. In late 2008, melamine-contaminated milk formula was confirmed to be associated with renal calculi in children in China.[3] Since then, altogether 15 577 children were screened at our hospital for renal stones. In this article, we describe the clinical features and risk factors of calculi in children fed with melamine-contaminated milk products.

Methods

Subjects

A total of 15 577 children fed with melamine tainted milk or infant formula were screened for urinary tract calculi in our hospital from September 12 to October 9, 2008. They were 7988 males and 7589 females, aged from 1 month to 180 months, with a mean of 22 months. Ultrasonography of the urinary tract system was performed in all these children. In children with urinary tract calculi, the liver and gall bladder were also scanned and urinalysis was performed.

Among 15 577 children, 846 with detailed data screened from September 17 to 25 were enrolled for further analysis. They were 429 males and 417 females, aged from 1 month to 5 years, with a median age of 18 months. These children were divided into calculus group (326 children) and non-calculus group (520 children) according to their results of the screening.

Weight, height, and head circumference of the children, type of milk formula (brands of milk formula, Sanlu brand formula defined as 1, Shengyuan as 2, Yashili as 3, Shien as 4, Yili as 5, and other brands as 6 according to the highest levels of melamine),[4] age of feeding with contaminated milk products or formula, duration of feeding with contaminated milk products, number of days after last feeding of milk formula, type of feeding (1, complete feeding with contaminated milk formula; 2, mixed feeding with contaminated formula and other food), water intake (1, minimal water intake; 2, moderate water intake, 2-4 times water intake a day; intake of a plenty of water, >5 times water intake a day except for milk), and laboratory data were recorded and analyzed. In children fed with several types of milk formula, the formula was defined to contain the highest concentration of melamine.

The weight, height, and circumference *Z* scores (SDSs) of the children were calculated according to the criteria set up in 1995 in China.[5] Complaints from the parents of 203 children with renal calculi and 94 children without calculi included red urine color, red small particles in urine, cry or discontinuance during urination, frequent diarrhea (more than 3 times in a year), and fever (more than 4 times a year).

Informed consent was obtained from the parents of

Table 1. The incidence of renal calculi in patients fed with different types of formula with different positive rates

Company and brand of milk formula	Highest level (mg/kg)*	Incidence of calculi (%)
1 Sanlu (Sanlu Limited Company, Shijiazhuan)	2563.00	15.7
2 Shengyuan (Shengyuan Dairy Limited Company, Qingdao)	150.00	8.9
3 Yashili (Yashili Limited Company, Shanxi or Guangdong)	53.40	7.3
4 Shien (Shien Infantile Nutriment Limited Company, Guangzhou)	17.00	4.5
5 Yili (Yili Limited Company, Neimenggu)	12.00	3.4

*: the highest concentration of melamine in formula.

Table 2. The characteristics of patients with or without calculi

	Calculus group	Non-calculus group	$t/Z/\chi^2$	*P* value
Gender (male/female)	131/195	298/222	0.518	0.471
Age (mon)	16.0 (1.0–60.0)	19.0 (1.0–60.0)	0.611	0.541
Number of cases apt to fever	23/203	7/94	1.067	0.302
Number of cases apt to diarrhea	13/203	0/94		0.011*
Red urine color or particles in urine	20/203	3/94		0.060*
Frequent micturition	38/203	9/94	4.034	0.045
Cry or discontinuance during urination	29/203	5/94	5.096	0.024
Birth weight (kg)	3.27±0.54	3.33±0.59	0.772	0.441
Weight SDS	0.39 (-4.33–3.22)	0.53 (-3.49–2.80)	1.978	0.048
Height SDS	0.57 (-2.63–4.81)	0.68 (-2.20–4.53)	0.394	0.694
Head circumference SDS	0.08 (-6.33–3.25)	0.85 (-1.33–2.38)	1.992	0.046
Duration of milk formula consumption (mon)	15.70±12.84	12.53±9.49	2.234	0.026
Age of formula started (mon)	0 (0–36.0)	2.0 (0–31.0)	1.976	0.048
Number of days between last formula consumption (day)	7.0 (1.0–2000.0)	7.0 (1.0–1200.0)	1.691	0.091
Type of feeding (milk/mixed)	82/116	30/64	2.433	0.119
Water intake (minimal/moderate/plenty)	6/98/90	0/31/60	11.012	0.004

*: Fisher's exact test.

all participants. The screening program was approved by the Ethics Committee of Zhejiang University School of Medicine.

Statistical analysis

Pearson's Chi-square test or Fisher's exact test was used to measure enumeration data between the two groups when possible. Quantitative data were expressed as mean ± SD or median (mix-max), and then analyzed with Student's *t* test or the Mann-Whitney *U* test. Logistic regression was used to analyze the risk factors for calculi in the urinary tract. A 2-tailed $P<0.05$ was considered statistically significant.

Results

In the 15 577 children, 562 had urinary tract calculi, with a positive rate of 3.61%. The incidence was increased with the increased concentration of melamine in the milk formula (Table 1), i.e., 15.7% in Sanlu, 8.9% in Shenyuan, 7.3% in Yashili, 4.5% in Shien, and 3.4% in Yili, and 0.4% in other brands.[4]

In 846 children with detailed data enrolled in this analysis (Table 2), micturition, cry or discontinuance during urination, and diarrhea were more frequent in the calculus group than in the non-calculus group ($P<0.05$, respectively), but the rate of red urine color or particles in urine showed a marginal difference ($P=0.060$). The weight SDS lower than 2 was found in 11 (5.73%) of 192 children in the calculus group and in 3 (3.26%) of 92 children in the non-calculus group. The head circumference SDS lower than 2 was found in 10 (5.21%) of 192 children in the calculus group but in no patients in the non-calculus group. Children in the calculus group had lower weight and head circumference SDSs than those in the non-calculus group ($Z=1.978$, $P=0.048$; $Z=1.992$, $P=0.046$; respectively). The duration of feeding with contaminated milk formula, age of feeding with milk formula, and water intake between the two groups were significantly different. The calculus group tended to have a long duration of feeding with the contaminated formula, early feeding with the formula, and minimal water intake ($t=2.234$, $P=0.026$; $Z=1.976$, $P=0.048$; $\chi^2=11.012$, $P=0.004$). Logistic regression showed that long duration of feeding with contaminated milk formula, feeding with high melamine content formula, and minimal water intake were independent determinants for calculi of the urinary tract ($P<0.05$, respectively), with a variance of 18.2% (Table 3).

Table 3. Logistic regression analysis of the risk factors for calculi

	Coefficient	SE	Wald	*P* value
Water drinking	-0.899	0.267	11.361	0.001
Type of formula	-0.180	0.086	5.743	0.017
Duration of formula intake (mon)	0.030	0.013	5.621	0.018

R^2 (Cox & Snell) = 0.182

In 326 children with calculi, the diameter of calculi ranged from 0.1 to 3.3 cm with a median of 0.2 cm. Most of them (281/326, 86.20%) had small calculi less than 0.5 cm in diameter. Multiple calculi were noted in 227 children (86.20%). Of the 326 patients, 122 (37.42%) had calculi on both sides of the urinary tract, 101 (30.98%) on the right side, and 103 (31.60%) on the left side. Ureteral calculi were noted in 14 children (4.29%) and bladder calculi in 4 (1.23%). Urinary tract distention observed in 34 children (10.43%) included hydronephrosis (26 children) and ureter distention (11). Diffuse renal lesions, renal failure and ascites were noted in 4, 3 and 2 children, respectively.

Urinalysis was performed in 147 of the 326 children with calculi. Increased WBC count was noted in 18 children (12.24%). Hematuria was found in 28 children (19.05%), including 14 children with macroscopical hematuria and 14 with microscopic hematuria. Occult blood was positive in 25 children (17.01%) and protein positive in 8 (5.44%). Increased blood creatinine (167-695 μmol/L; normal range, 22-97 μmol/L) was seen in 3 children. Abnormalities of the liver in 5 children with calculi included hepatomegaly (2 children), elevated aspartate aminotransferasemia (2), and gallstone (2).

Sodium bicarbonate (intravenous injection of 5% sodium bicarbonate 2-3 ml/kg for inpatients, 30-50 mg/kg tablet for outpatients) and Chinese traditional medicine (*Herba Lysimachie* granules for oral administration) were given to the patients, and antibiotics were administered to those with urinary tract infection. Forty-one children with calculi over 0.5 cm in diameter or with symptoms of urinary obstruction and/or renal failure were admitted to our hospital from September 12 to 31, 2008. Calculi were removed operatively in 5 children with acute renal failure or serious urinary obstruction. Renal function of these 5 children recovered within one week. Calculi in the other 36 children minimized. They were hospitalized for 8 to 34 days with a median of 11 days. Fifty-four patients with calculi less than 0.5 cm in diameter were treated by outpatient visits. One month later, the 54 outpatients were rechecked and the calculi disappeared in 49 (90.74%). Calculi in the other 3 children minimized and in 2 unchanged.

Discussion

Melamine contaminated milk products resulted in calculi in the urinary system in infants and children

in China in 2008.[6-8] The highest concentration of melamine in the formula was 2563.0 mg/kg, significantly higher than the limitation of the WHO recommendation (a tolerable daily dose of 0.2 mg per kilogram of body weight for melamine) and FDA recommendation (2.5 part per million of melamine in food and 1 part per million in infant formula).[8]

In animal models, 90% of melamine consumed was excreted in urine after 24 hours.[9,10] Calculus formation appears to be melamine concentration dependent. Altogether 562 (3.61%) children were found to have urinary tract calculi in our hospital, and over 290 000 throughout China. The rate is significantly higher than that in Hong Kong (0.03%), showing that urinary tract calculi are closely associated with melamine concentration, and that the tainted milk products in Hong Kong had a lower melamine concentration.[6] On screening, most children with calculi were asymptomatic. Except urinary tract calculi, change of urine color, particles in urine, frequent micturition, cry or discontinuance when urination, even anuria and swelling were noted in few children. These findings are similar to those reported in animal experiments.[11,12]

We found the following characteristics of the urinary calculi in most children: (1) multiple and small calculi or "sediment-like calculi"; (2) calculi located in the bilateral collection system, followed by the ureter, renal pelvis, renal calices, and bladder; (3) calculi with a trailing edge detected with a pallid acoustic shadow on ultrasonography;[13] (4) calculi containing melamine and uric acid negative for X-ray, which can be differentiated from other kinds of calculi (e.g., calcium oxalate calculi).[14]

Consistent with our hypothesis, higher melamine concentrations in milk products (e.g., Sanlu formula), longer duration of melamine consumption, and minimal water intake are the risk factors for renal stones and deposits. In our screening, a child fed with Sanlu formula for 42 days had bilateral multiple calculi over 0.5 cm in diameter. Hence, all children consumed melamine tainted milk products should undergo ultrasonography and urinalysis on screening.

The toxicity of melamine in humans is unclear. Histopathological investigation on animals showed unique polarizable crystals in distal tubules or collecting ducts, which were attributed to secondary oxalosis associated with tubular injury. In acute cases, the distal tubules exhibited necrosis with initial multifocal mild acute neutrophilic tubular inflammation and incorporation of some crystals into the tubular wall because of epithelial overgrowth. In chronic cases, crystals were larger with associated chronic lymphoplasmacytic to granulomatous tubulointerstitial inflammation and fibrosis.[1,2,11] The small crystals in the collecting duct and secondary lesion might be the reason why some animals had acute renal failure without visible calculus on sonography.[1,2] Some extrarenal lesions attributed to uremia (e.g., oral ulcers along the ventrolateral aspect of the tongue, mineralization of gastric mucosa, pulmonary smooth muscle and alveolar walls) and low nutrition milk (e.g., anemia, vomiting, diarrhea and retarded development) were reported.[1] In this study, we noted that patients with renal calculi had lower weight and head circumference SDSs. This finding is consistent with the fact that melamine-contaminated milk formula had lower nourishments including protein, and indicated that feeding of such formula might affect the brain development in infants and children. Moreover, we also observed abnormalities of the liver in 5 children with renal calculi. The abnormalities may be related to melamine contamination in milk products.

Animal studies showed that polyunsaturated fatty acids might reduce the development of tumors by modulating pre-neoplastic urothelial proliferation induced by melamine.[15,16] No data are available on the therapy for humans. We have used conservative treatment including rehydration, alkalification of urine, and Chinese traditional medicine to eliminate renal calculi. To children with urinary tract infection, antibiotics with low renal toxicity were given for 1-2 weeks. Children with stone obstruction, abnormal renal function or severely abnormal urea outcome should be hospitalized for further investigation. Small calculi can be eliminated without further treatment in both outpatients and inpatients. An 11-month girl had a 0.5-cm calculus excreted from the urethra itself 3 days after hospitalization. However, case control study of large cohort may be needed to investigate the effectiveness of these managements.

In children with acute renal failure, life-threatening situations (e.g., hyperkalemia, hypertension) must be corrected as soon as possible with the application of sodium bicarbonate, insulin, blood purification, hypotensive drug, peritoneal dialysis, and other methods although acute renal failure is rare. If necessary, surgical intervention should be done to remove stone obstruction as soon as the condition is permitted. As renal calculi comprise acidum uricum and melamine, which are not solid,[2] extracorporeal shock-wave lithotomy is not recommended. We removed renal calculi operatively in 5 children. After operation, their renal function returned to normal. Studies revealed simple urothelial hyperplasia, dysplasia, and carcinoma in animals chronically treated with melamine.[16-18] It is not known whether human urinary tract tumors share a similar molecular mechanism. Long-term follow-up is required.

Early in 2004, at least 12 babies in China's eastern Anhui province died after consumption of milk products.[19] Such sporadic events prompt us to consider the safety of food in China and how should we establish an early surveillance warning system.

There are some limitations of this study, for example, there was no control group in this study, especially in analysis of weight, height and head circumference, and we did not record the data in detail on all children. Only part of the children participating in the screen were enrolled in the analysis. Data for the calculus and non-calculus groups were not complete and were taken from most of the them without follow-up.

In summary, melamine-contaminated milk products cause urinary tract calculi which can be treated symptomatically with good outcomes. Extensive and long-term follow-up of children who consumed melamine tainted milk products is required.

Acknowledgements

We thank all the children and their parents for allowing the data in this research project. We also thank the staff of department of ultrasound and nephrology for their patient care and organization.

Funding: None.
Ethical approval: This study was approved by the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine.
Competing interest: None.
Contributors: Zhang L and Zou CC were responsible for protocol development, preliminary data analysis, and writing of the manuscript. Zhao ZY supervised the design and execution of the study. Wu LL, Wang YP and Liu AM were responsible for patient screening.

References

1 Brown CA, Jeong KS, Poppenga RH, Puschner B, Miller DM, Ellis AE, et al. Outbreaks of renal failure associated with melamine and cyanuric acid in dogs and cats in 2004 and 2007. J Vet Diagn Invest 2007;19:525-531.
2 Puschner B, Poppenga RH, Lowenstine LJ, Filigenzi MS, Pesavento PA. Assessment of melamine and cyanuric acid toxicity in cats. J Vet Diagn Invest 2007;19:616-624.
3 Ministry of health of the People's of Republic of China. [http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohbgt/s3582/200809/37769.htm].
4 Ministry of health of the People's of Republic of China. [http://www.aqsiq.gov.cn/zjxw/zjxw/zjftpxw/200809/t20080916_89958.htm].
5 Hu YM, Jiang ZF. Development. In: Hu YM, Jiang ZF, eds. Zhu Futang Textbook of Pediatrics. Beijing: Chinese Pepole's Health Publisher, 2002: 11-66.
6 Lam HS, Ng PC, Chu WC, Wong W, Chan DF, Ho SS, et al. Renal screening in children after exposure to low dose melamine in Hong Kong: cross sectional study. BMJ 2008; doi:10.1136/bmj.a2991.
7 Xin H, Stone R. Tainted milk scandal. Chinese probe unmasks high-tech adulteration with melamine. Science 2008; 322:1310-1311.
8 Ingelfinger JR. Melamine and the global implications of food contamination. N Engl J Med 2008;359:2745-2748.
9 Mast RW, Jeffcoat AR, Sadler BM, Kraska RC, Friedman MA. Metabolism, disposition and excretion of [14C]melamine in male Fischer 344 rats. Food Chem Toxicol 1983;21:807-810.
10 Baynes RE, Smith G, Mason SE, Barrett E, Barlow BM, Riviere JE. Pharmacokinetics of melamine in pigs following intravenous administration. Food Chem Toxicol 2008;46: 1196-1200.
11 Thompson ME, Lewin-Smith MR, Kalasinsky VF, Pizzolato KM, Fleetwood ML, McElhaney MR, et al. Characterization of melamine-containing and calcium oxalate crystals in three dogs with suspected pet food-induced nephrotoxicosis. Vet Pathol 2008;45:417-426.
12 Cianciolo RE, Bischoff K, Ebel JG, Van Winkle TJ, Goldstein RE, Serfilippi LM. Clinicopathologic, histologic, and toxicologic findings in 70 cats inadvertently exposed to pet food contaminated with melamine and cyanuric acid. J Am Vet Med Assoc 2008;233:729-737.
13 Jiang GP, Zhao ZY, He J. The characteristics of melamine-related renal stones by ultrasound. In Press 2009. World J Pediatr.
14 Ministry of health of the People's of Republic of China. [http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohyzs/s3586/200809/37772.htm].
15 Cremonezzi DC, Diaz MP, Valentich MA, Eynard AR. Neoplastic and preneoplastic lesions induced by melamine in rat urothelium are modulated by dietary polyunsaturated fatty acids. Food Chem Toxicol 2004;42:1999-2007.
16 Cremonezzi DC, Silva RA, del Pilar Diaz M, Valentich MA, Eynard AR. Dietary polyunsatured fatty acids (PUFA) differentially modulate melamine-induced preneoplastic urothelial proliferation and apoptosis in mice. Prostaglandins Leukot Essent Fatty Acids 2001;64:151-159.
17 Ogasawara H, Imaida K, Ishiwata H, Toyoda K, Kawanishi T, Uneyama C, et al. Urinary bladder carcinogenesis induced by melamine in F344 male rats: correlation between carcinogenicity and urolith formation. Carcinogenesis 1995;16:2773-2777.
18 Melnick RL, Boorman GA, Haseman JK, Montali RJ, Huff J. Urolithiasis and bladder carcinogenicity of melamine in rodents. Toxicol Appl Pharmacol 1984;72:292-303.
19 [http://bgt.aqsiq.gov.cn/tpxw/ywbd/200610/t20061027_13257.htm].

Received December 2, 2008
Accepted after revision December 31, 2008