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Prevalence of melamine exposure in Hong Kong children

在這項研究中，我們共化驗了502個尿液樣本。這些尿液樣本是從一項於2007-08年度進行的全港性系統學童評估中收集而來的。該項評估共有2119名香港華裔學童參與，他們是隨機從5間小學（804人）和6間中學（1315人）中邀請參加的。基於已發表的文獻，我們定義尿液三聚氰胺肌酸酐比例高於7.1 μg/mmol為高尿液三聚氰胺水平。我們聯絡及邀請被檢測有高尿液三聚氰胺水平之學童於2009年回來作臨牀評估（包括尿液分析及超聲波檢查）。這項研究共發現9%（47/502人）學童有高尿液三聚氰胺水平。一名學童拒絕回來檢查。46名回來檢查的學童中（平均±標準偏差年齡為13.9±2.9歲；28%男生），所有學童的泌尿系統超聲波檢查都是正常的。他們的奶類食品使用記錄跟尿液中三聚氰胺水平也沒有任何相關係。基於這項短期跟蹤研究（中位數為23.5月），香港華裔學童被檢測有高尿液三聚氰胺水平都健康良好，並沒有不良或嚴重的後遺症。

Key Messages

1. About 9% of Hong Kong school children had elevated urine melamine/creatinine ratios of >7.1 μg/mmol.
2. There was no association between milk consumption and urinary melamine levels.
3. Hong Kong school children with high urine melamine levels appeared to have benign clinical course.

Introduction

In early September 2008, melamine-tainted milk products in Mainland China raised public concerns about food safety.[1] Melamine is widely used in plastics, dishware, laminates, glues, and toy coatings. It is a potential food contaminant and a public health hazard. Excessive melamine exposure has been sporadically reported in Hong Kong. The incidence of renal involvement (echogenic renal foci) has been reported to be 0.03% to 0.6%, depending on selection criteria and evaluation methods.[2-4] From 2007 to 2008, we conducted a territory-wide survey to examine the prevalence of this metabolic syndrome in Hong Kong youths. Using archived urine samples, we assayed the urine melamine levels in a sub-cohort and evaluated their clinical status after 2 years. We also determined the correlation between urinary melamine levels and daily milk consumption.

Methods

This study was conducted from April 2009 to March 2011. Stored urine aliquots were collected from a territory-wide cohort surveyed in 2007 to 2008. A total of 2119 Hong Kong Chinese school children (67% girls) aged 6 to 20 years from five primary schools (804 children) and six secondary schools (1315 children) were randomly selected using a cluster sampling method. Informed consents were obtained from both the participants and their parents or guardians. The study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. In a validated one-minute dietary questionnaire, participants' habit of milk consumption was inquired using a question "Are you drinking more than one cup of milk per day?" Spot morning urine specimens were collected from all agreed participants during the field study for measurement of the albumin/creatinine ratio (ACR). Albuminuria was defined as an ACR of >3.5 μg/mmol. A total of 502 urine aliquots were assayed for melamine level. High urine melamine level was defined as a urine melamine/creatinine ratio of >7.1 μg/mmol.[5] Subjects with high urine melamine level were invited for clinical evaluation in 2009 including urinalysis and ultrasound imaging of the urinary system. Renal ultrasonography was performed by an experienced radiographer using the Philips ATL HDI5000 ultrasound machine. Both kidneys were evaluated for the presence of renal stones, hydronephrosis or related renal scarring.

Urine melamine was measured by a liquid chromatography tandem mass spectrometry method. An aliquot of 20 μL of urine was added to 200 μL acetonitrile (ACN) containing 10 ppb stable isotope labelled melamine

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internal standard ($^{13}C_3$, $^{15}N_3$-melamine, Cambridge Isotope Laboratories, Andover, MA, USA). The solution was vortex mixed before centrifugation at 16 000 g for 5 minutes and the clear supernatant was transferred to a sample vial. Measurement was performed on a UPLC Waters Xevo TQ System (Waters, Milford, MA, USA). Calibrators (10-1000 μg/L) were prepared by spiking appropriate amounts of melamine into a negative pooled urine sample. An aliquot of 5 μL of calibrators/extracted melamine was injected into an ACQUITY UPLC BEH HILIC column (2.1x150 mm, 1.7 μm), which was kept at 45ºC. Weak and strong wash solutions for UPLC were ACN and water, respectively. We used 50% ACN in water as the seal wash solution. Melamine and the internal standard were separated from matrix interference by a gradient programme using mobile-phase solutions of 10 mM ammonium acetate in water and 10 mM ammonium acetate in 97% ACN in water at a flow rate of 500 μL/min. For the mass analyser, capillary voltage was optimised at 3.9 KV, cone voltage at 40 V, and collision energy at 24 V. The source and desolvation temperatures were at 150ºC and 500ºC, respectively. Positive electrospray ionisation tandem MS analyses were performed using *m/z* of 127 to 185 and 127 to 168 as quantitative and qualitative MRMs for melamine, respectively; and *m/z* of 133 to 189 as the MRM for the internal standard. The dwell time for each MRM was 50 msec. Both melamine and the internal standard eluted at around 3.5 minutes. Additional mobile-phase gradient programming was used to remove matrix interference and recondition the column for the next analysis. Injection-to-injection time was 6 minutes. Quantitation was performed by the TargetLynx Manager of the Waters MassLynx 4.1 software. The limit of quantitation was 5 μg/L and the linearity was up to 10 000 μg/L. Between-batch precision coefficients of variation for quality control samples (10, 100, 400 and 1000 μg/L) were <10%. Recoveries for spiked standards into blank matrix at concentrations of 100, 400, and 1000 μg/L were >99%.

Urine albumin and creatinine were measured on a Roche Modular Analytics system (Roche Diagnostics GmbH, Mannheim, Germany). Urine albumin was measured by turbidimetry and urine creatinine by the kinetic Jaffe reaction using standard reagent kits provided by the instrument manufacturer. Their analytical performances were within the manufacturer's specifications.

Urinalysis was performed by Multistix (Siemens urine test strips 10SG, Bayer). Microscopic examination was performed, by examining 60 μL of urine in microtitre plates using an inverted microscope, to look for red blood cells, casts, and crystals in urine. Quantitative culture of urine was performed on a chromogenic medium (CPS ID3 [Biomerieus] plate using 10 μL standard loops incubated aerobically for 18 to 24 hours at 35ºC.

Baseline characteristics in subjects with and without high urinary melamine levels (urine melamine/creatinine ratio, >7.1 μg/mmol) were compared using the Pearson's Chi square test, Fisher's exact test, T-test, and Mann-Whitney *U* test, as appropriate. Association between daily milk consumption and melamine level was assessed using Mann-Whitney *U* test and Pearson's Chi-square test, depending on the data format of the melamine level. The Spearman correlation coefficient was used to assess the correlation between urine ACR and the urine melamine/creatinine ratio. All statistical tests were two-sided, and a P value <0.05 was considered statistically significant.

Results

Table 1 shows the baseline characteristics of the 502 school children recruited in 2007 to 2008. The median spot urine melamine/creatinine ratio was 0.8 (range, undetectable to 1467) μg/mmol; in 213 (42%) the ratio was undetectable (zero). Urine albumin ranged from undetectable to 207 μg/mmol; the median urine ACR was 0.70 (interquartile range, 0.45-2.01) μg/mmol. Age, body weight, body height, and body mass index were associated with elevated urinary melamine levels (Table 1). There was no significant correlation between the urine melamine/creatinine ratio and ACR. There were 25 subjects with missing milk consumption data. In the 477 subjects with available data, no significant correlation was noted between the milk consumption and urine analysis (Table 2).

Of 47 (9%) subjects with high urine melamine levels, 46 (28% boys; mean±standard deviation age, 13.9±2.9 years) were followed up in 2009 (Table 3). The median follow-up duration was 23.5 (interquartile range, 19.8-30.6) months. None had any abnormality in the urinary system based on ultrasonography. None recalled any significant urinary tract symptoms or had abnormalities on urinalysis.

Discussion

In agreement with a previous report of children aged ≤12 years who consumed milk products contaminated with melamine,[2] this study also did not detect any major adverse renal outcomes in older school children with elevated urine melamine level. There was no association between the urine albumin level and the melamine level in subjects with high urine melamine levels.

In this cohort, young age, low body weight and height, low body mass index were all associated with elevated urinary melamine level. As younger school children tend to have higher milk consumption, age may be a factor linking urinary melamine level and anthropometric parameters, although there was no significant correlation between milk consumption and urinary melamine level, probably due to the small sample size. In young children who were not breast-fed, milk products, particularly the powdered infant formula, are the major sources of nutrients in infants. In 2008, the discovery of melamine contamination of these milk products in Mainland China raised alarm in the

Table 1. Baseline characteristics of the study sample (n=502) in 2007 to 2008

Characteristics	All (n=502)	Urine melamine/creatinine ratio of >7.1 μg/mmol		P value (test)
		No (n=455)	Yes (n=47)	
No. (%) of males	167 (33.3)	153 (33.6)	14 (29.8)	0.595 (Chi square test)
No. (%) of females	335 (66.7)	302 (66.4)	33 (70.2)	
Mean±SD age (years)	13.2±3.0	13.3±3.0	12.0±2.8	0.004 (T-test)
Mean±SD body weight (kg)	43.6±11.8	44.1±11.7	39.3±12.1	0.008 (T-test)*
Mean±SD body height (cm)	152.3±13.6	152.8±13.4	147.4±14.6	0.010 (T-test)*
Mean±SD body mass index (kg/m^2)	18.5±3.0	18.6±3.0	17.5±2.4	0.026 (T-test)*
Weight status (No. [%] of participants)				0.193 (Fisher's exact test)
Normal	428 (85.3)	384 (84.4)	44 (93.6)	
Overweight	51 (10.2)	48 (10.5)	3 (6.4)	
Obesity	23 (4.6)	23 (5.1)	0	
Median (IQR) urine albumin/creatinine ratio (μg/mmol)	0.70 (0.45-2.01)	0.69 (0.44-3.23)	0.70 (0.50-1.39)	0.820 (non-parametric Mann-Whitney *U* test)
Median (IQR) urine melamine/creatinine ratio (μg/mmol)	0.76 (0-2.62)	0.52 (0-1.73)	13.21 (9.09-21.55)	<0.001 (non-parametric Mann-Whitney *U* test)

* Not significant difference after adjusting for age

Table 2. Association between urine melamine level and daily milk consumption

Parameter	Drinking more than a cup of milk per day*		P value (test)
	No (n=378)	Yes (n=99)	
Median (IQR) urine melamine (μg/L)	6.0 (0-25.0)	11.0 (0-33.0)	0.280 (Mann-Whitney *U* test)
Median (IQR) urine melamine/creatinine ratio (μg/mmol)	0.62 (0-2.33)	1.00 (0-3.19)	0.182 (Mann-Whitney *U* test)
Urine melamine/creatinine ratio of >7.1 μg/mmol (No. [%] of subjects)			0.072 (Chi square test)
No	347 (91.8)	85 (85.9)	
Yes	31 (8.2)	14 (14.1)	

* 25 subjects with missing milk-drinking data

Table 3. Results of 46 subjects with urine melamine/creatinine ratio of >7.1 μg/mmol in 2009

Parameter	Result
No. (%) of males	13 (28.3)
No. (%) of females	33 (71.7)
Mean±SD age (years)	13.9±2.9
Mean±SD body weight (kg)	47.9±15.3
Mean±SD body height (cm)	155.9±11.8
Mean±SD body mass index (kg/m^2)	19.7±4.5
Urine total protein (g/L) [No. (%) of subjects]	
<0.1	35 (77.8)
0.1-0.2	6 (13.3)
0.2-0.3	2 (4.4)
0.3-0.4	0
≥0.4	2 (4.4)
Median (IQR) urine albumin/creatinine ratio (μg/mmol)	0.70 (0-2.55)
No. (%) of abnormal ultrasound finding on the urinary system	0

international community. According to the Ministry of Health of China, since 21 September 2008, almost 40 000 children had consumed melamine-tainted milk, with 13 000 hospitalisations and acute renal failure in 104 children.[1] More than 52 000 children and infants had sought medical treatment in Mainland China, with four reported deaths.

As melamine is not metabolised and rapidly eliminated in the urine of cats and dogs, melamine and its structural analogues, such as cyanuric acid, may interact and form melamine-cyanurate crystals. Human and primates have much higher uric acid concentrations in the blood, and melamine-urate crystals are more likely to form. Animals fed with melamine developed kidney stones causing urinary tract obstruction. Deaths secondary to urinary tract stones and acute renal failure in infants and young children exposed to very high melamine levels for prolonged period have been reported. Melamine is also widely used in plastics, dishware, adhesives, and toy coatings. Thus, ingestion of melamine as environmental pollutants may be a silent health hazard in children.

In our study, selection bias was unlikely owing to the random nature of the school sampling and the territory-

wide survey having been conducted before the melamine-tainted formula became an issue. Thus, potential recall bias in reporting milk consumption was not likely and might well reflect the real picture of melamine ingestion from food and environmental pollution. In previous reports of renal incidents,[2-4] subjects with known consumption of melamine-contaminated milk products or referred from designated outpatient clinics to special assessment centres were studied. These subjects might have had symptoms or their parents might have had increased alertness to seek medical attention. Our study had the advantage of being capable of identifying subclinical/silent cases missed in previous studies.

There are several limitations in our study. First, only one random spot urine specimen was collected. School children with high melamine levels might have transient exposure to melamine-tainted food products. Second, whether prolonged or repeated exposure to consumption of melamine-contaminated food might lead to long-term adverse renal outcome cannot be addressed by this short-term study. Third, the sample size was relatively small (502 children). Fourth, a detailed food diary was not recorded when the urine was sampled in 2007 to 2008.

Conclusions

About 9% of the study cohort had elevated urinary melamine level. There was no association between milk consumption and urinary melamine levels. In this short-term follow-up study, Hong Kong Chinese school children with high urine melamine levels appeared to have a benign clinical course. Longer follow-up is required to detect any long-term adverse clinical effects.

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