HK J Paediatr (new series) 2012;**17**:12-23

One-year Follow Up of Melamine-associated Renal Stones in Sichuan and Hong Kong

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Abstract

Background: An outbreak of melamine-associated renal stones occurred in mainland China in 2008, which led to large scale community screening of renal stones in Hong Kong. We hypothesised Hong Kong children screened positive did not suffer from melamine-associated renal stones. ***Methods:*** This one-year follow-up study compared the clinical features, renal ultrasound findings, and urinary interleukin 8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1) to urinary creatinine ratios between 44 Sichuan children admitted to hospital for melamine-associated renal stones and 22 Hong Kong children screened positive with renal stones. ***Results:*** The mean age (±SD) in months of the Sichuan children (25.7±23.8) was significantly lower than that of Hong Kong children (75.0±42.1, p<0.0001). The melamine concentration in the milk consumed by the Sichuan children was significantly higher than that by Hong Kong children. All 44 Sichuan children and only 15 of the 22 Hong Kong children consumed melamine-tainted milk product (MTMP). All except one of the 15 Hong Kong children had their daily melamine

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Received July 18, 2011

intake below the WHO tolerable daily intake of 0.2 mg melamine/Kg body weight. At diagnosis, the median number of stones and the mean largest stone size in the Sichuan children (4, 6.3 mm) were significantly higher than that in Hong Kong children (1, 3.8 mm, p<0.01). By 12 months follow-up, 28% and 48% of the Sichuan and Hong Kong children still had renal stones respectively. Urinary IL-8/creatinine ratio was significantly higher in Sichuan children with stones than other groups at 6 and 9 months follow-up. By 12 months follow-up, no such difference was observed among any groups. The above findings did not differ significantly whether the comparison with the Sichuan children was with the whole cohort of 22 Hong Kong children or the 15 who had consumed MTMP. In contrast, the 2 groups of Hong Kong children with and without MTMP consumption were similar in their demographics and clinical features. ***Conclusions:*** Hong Kong children had significantly different clinical features including MTMP consumption as compared to Sichuan children, suggesting Hong Kong children did not suffer from melamine-associated renal stones.

Key words Cytokines; Interleukin-8; Melamine; Milk; Renal stones

Introduction

In September 2008, melamine was reported to be added to infant formula in China to elevate protein content apparently, which resulted in a massive outbreak of melamine-associated renal stones in children.[1] According to a report from the Chinese Ministry of Health in December 2008, 22,384,000 children with suspected melamine exposure were examined with 294,000 diagnosed to have urinary stones and 51,900 hospitalised.[2] In our previous study, we identified 189 (2.6%) of 7,328 children who presented to West China Second University Hospital (WCSUH) in Sichuan with melamine exposure to have ultrasound findings of urinary stones.[3] Melamine-associated urinary stones were shown to be more frequent in infants and more severe in children from poorer families. Repeat ultrasound for 51 children at 15.3±8.9 days follow-up revealed 33 (64.7%) discharged all the stones.[3] However, long-term follow up studies are still infrequent. 89.9% of 46 children in Shijiazhuang with such stones identified in a population-based screening and follow-up study had their stones discharged at 6-month follow-up,[1] while in a hospital-based study in Beijing, 95.5% of 265 children with melamine-associated urolithiasis had their stones discharged at 12-month follow-up.[4]

In response to this outbreak, the Hong Kong Government formed an ad hoc Expert Group in Melamine Incident to advise her in all aspects including clinical care of suspected cases, public health response, research and food safety. According to the Group's third and final report,[5] a total of 56,847 children were screened in the Designated Clinics. Of these, 27,616 were referred to the Special Assessment Centres where further tests including renal ultrasounds were done. Of these, 15 children with renal stones suspected to be related to melamine-tainted milk product (MTMP) were reported to Centre for Health Protection (CHP). Of these, 6 were classified as "probable" and 9 "suspected". Their median age is 6 years (range 30 months to 12 years). There could be other suspected cases not reported to CHP and the long-term outcome is still unknown.

The mechanism of renal damage caused by melamine is thought to be due to primarily obstructive uropathy, resulting in acute renal failure in the most severe cases.[6-10] Histopathological features of the kidney after acute renal failure from melamine showed generalised lymphocytic infiltration and fibrosis within the renal interstitium and glomeruli, with swollen tubular cells and crystals within the tubular lumen.[11] We and others have shown such urinary stones comprised of melamine, uric acid and metal phosphates but without cyanuric acid.[3,11] Whether these crystal stones could induce inflammation and for how long is still unknown. Inflammatory cytokines interleukin-8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1) were reported to associate with crystal-induced inflammation of kidneys in human and animal studies.[12] Whether these two inflammatory cytokines can be used to monitor inflammation of kidneys caused by these melamine renal stones is not known.

In the present study, to test the hypothesis that the Hong Kong children screened positive to have renal stones with ultrasound by the Special Assessment Centres did not suffer from melamine-associated renal stones, we compared their clinical features, renal ultrasound findings and urinary IL-8/creatinine and MCP-1/creatinine ratios with those of Sichuan children admitted to hospital with melamine-associated renal stones.

Methods

Patients

The WCSUH is a teaching hospital in Chengdu, Sichuan. Between 13 September and 15 October 2008, 7,328 children presented to the WCSUH with a concern of melamine-tainted milk product (MTMP) associated urinary stones. Of these, 189 children had ultrasound findings of urinary stones and 51 of them were admitted for treatment because of symptoms or severe ultrasound abnormalities.[3] They were examined by B-mode ultrasound with a probe of 3.5 to 5 megahertz. The surveillance case definition of MTMP associated urinary stones is that issued by the World Health Organisation (WHO).[13] We included the 51 children admitted to hospital in our study because they were followed up and represent the more severe end of the spectrum of melamine-associated renal stones. Forty-four of these 51 Sichuan children admitted to WCSUH (Group A) were recruited for the follow-up of one year in the present study, with 7 not joining the study as the parents refused. The protocol was approved by the local ethical committee (WCSUH IRB). They were followed up with renal ultrasound and their urine samples were collected for determining urinary IL-8 and MCP-1 at around 6, 9 and 12 months after the renal stones had been defined initially.

A total of 34 children suspected to have melamine-associated renal stones were identified by the Special Assessment Centres in the Departments of Paediatrics and Adolescent Medicine of Pamela Youde Nethersole Eastern Hospital, Queen Elizabeth Hospital, Kwong Wah Hospital, United Christian Hospital, Tuen Mun Hospital and Queen Mary Hospital by the responsible paediatricians after screening 16,567 children under 12 years old using renal ultrasound. Of these, 22 children were recruited to join the present study (Group B), with the remaining 12 children not joining the study as the parents refused. These 22 children had renal ultrasound as scheduled by their paediatricians and their urine samples were collected for determining urine IL-8 and MCP-1 at around 6, 9 and 12 months as well. As renal ultrasound and urinary sample collections were done separately and not at the same date, classification of presence and absence of stones for the urinary samples will follow the result of the last previous ultrasound done. These 22 children were divided into the 15 who had consumed MTMP (Group C) and the 7 who did not (Group D) for further analysis. The research protocol was approved by the Institutional Review Board of The University of Hong Kong/Hong Kong West Cluster, Kowloon West Cluster Clinical Research Ethics Committee, Kowloon Central / Kowloon East Research Ethics Committee, Hong Kong East Cluster Ethics Committee and New Territories West Cluster Clinical & Research Ethics Committee.

Ultrasound Findings

Ultrasound abnormalities in the Sichuan children were classified using methods as we described before.[3] The number, size (the larger diameter) and location of the urinary stones were recorded. Aggregate stone index (ASI) is the summation of the sizes of all the kidney stones identified by renal ultrasound in a patient. For example, a patient with 2 stones identified with size 5 mm and 6 mm will have an ASI of 11 mm. The size of the largest stone in a patient will also be used for analysis. For the Hong Kong children, the renal ultrasound was scheduled by their paediatricians and the formal reports of these renal ultrasound were retrieved and abnormalities classified as for the Sichuan children.

Urine IL-8 and MCP-1 Measurement

Mid-stream urine was collected from the first morning void from children whose parents consented to join our study. Urinary inflammatory cytokines IL-8 and MCP-1 were measured by human IL-8 and MCP-1 DuoSet ELISA kits according to manufacturer's instruction respectively (R&D Systems, Minneapolis, MN). The urinary cytokine levels were adjusted with urinary creatinine levels measured in the same sample and expressed as cytokine/creatinine ratio.

Statistical Analysis

The Kaplan-Meier curve was used to show the discharge rate of renal stone in Sichuan and Hong Kong children during follow-up. Log-rank test was used to detect any significance in renal stone discharge rate between Sichuan and Hong Kong children during follow-up. Unpaired t-test was used to detect any significance in age difference between Sichuan and Hong Kong children. Chi-square test was used to detect any significant difference in sex between Sichuan and Hong Kong children. Wilcoxon rank sums test was used to detect any significant difference in ASI, stone number and largest stone between Sichuan and Hong Kong. One way ANOVA with post hoc Tuckey's test was used to detect any significant difference in IL-8 and MCP-1 levels among Sichuan and Hong Kong children who have stone and without stone at 6-month follow-up. Pearson correlation was used to detect the correlation coefficient of $\log_{10}$ IL-8 with largest stone size and stone number in Sichuan and

Hong Kong children at 6-month follow-up. All analyses and graphics were conducted by SAS software, v9.1 and GraphPad Prism v4.03. A significant level of $p<0.05$ was used for all analyses.

Results

Table 1 shows the age and sex of the 44 Sichuan and 22 Hong Kong children recruited in this study. The Sichuan children were significantly younger than the Hong Kong children with similar sex distribution, whether the Hong Kong children were analysed as a whole group or as subgroups of those with and without MTMP consumption. Similarly, the ASI and the number of stones were significantly higher in the Sichuan children as compared to that in Hong Kong children at presentation. In contrast, there were no difference in these parameters between the Hong Kong children with and without MTMP consumption. Table 2 shows there was no difference in the above parameters between these 44 recruited Sichuan children and the original whole cohort of 51.[3] Table 3 shows the melamine concentration in the milk consumed by Sichuan children was significantly higher than that in Hong Kong children ($p<0.0001$). Table 4 shows the degree of melamine intake of the 15 Hong Kong children with MTMP consumption. Table 5 shows the characteristics of stone at diagnosis between children with their stones passed out and not passed out completely were significantly different among the Sichuan children but not among the Hong Kong children.

Figure 1 shows the Kaplan-Meier curve of the presence of renal stones in Sichuan and Hong Kong children. At 12-month follow-up, about 28% of Sichuan and 48% of Hong Kong children still had ultrasound evidence of renal stones. The difference did not reach significance ($p=0.1302$). The Kaplan-Meier curve for the 15 Hong Kong children with MTMP consumption is similar to that of the whole cohort of 22. Two Sichuan children had their renal stones discharged but with hydronephrosis at 9 and 12-month follow-up respectively. One Hong Kong child had a linear echogenic focus with double line configuration which may suggest vascular calcification rather than renal stone was classified as having stone according to the definition of echogenic focus as equivalent to stone.

Table 1 Demographics of, stone size and number in children from Sichuan and Hong Kong at presentation

	Sichuan patients (n=44) Group A	**Hong Kong patients (n=22) Group B**	**Hong Kong patients with MTMP (n=15) Group C**	**Hong Kong patients without MTMP (n=7) Group D**	**Group A vs Group B p-value**	**Group A vs Group C p-value**	**Group A vs Group D p-value**	**Group C vs Group D p-value**
Age (months)					**<0.0001**[@]	**<0.0001**[@]	**0.0015**[@]	0.2208[@]
Mean	25.7	75.0	82.6	58.6				
SD	23.8	42.1	46.7	25.9				
Sex, n (%)					0.542[#]	0.9434[#]	0.1436[#]	0.2042[#]
Male	23 (52.3%)	9 (40.9%)	8 (53.3%)	1 (14.3%)				
Female	21 (47.7%)	13 (59.1%)	7 (46.7%)	6 (85.7%)				
ASI (mm)					**<0.0001**[@]	**<0.0001**[@]	**0.0028**[@]	0.2219[@]
Mean	18.2	4.3	4.65	3.68				
SD	12.1	1.7	1.79	1.36				
Largest stone size (mm)					**0.0087**[@]	0.0552[@]	0.0595[@]	0.2141[@]
Mean	6.3	3.8	4.11	3.19				
SD	4.2	1.6	1.68	1.32				
Stone number					**<0.0001***	**0.0004***	**0.0091***	0.9452*
Median	4	1	1	1				
Minimum	1	1	1	1				
Maximum	8	2	2	2				

[@]Unpaired t-test; [#]Chi-square test; *Wilcoxon rank sum test

ASI : aggregate stone index; MTMP: melamine-tainted milk product; SD: standard deviation

Table 2 Demographics of, stone size and number in 44 Sichuan children recruited in the current study compared to that in the original cohort of 51[3]

	Sichuan in-patients in current study (n=44)	Original cohort of Sichuan in-patients (n=51)	p-value
Age (months)			0.9023[@]
Mean	25.7	25.1	
SD	23.8	23.6	
Sex, *n* (%)			0.9602[#]
Male	23 (52.3%)	28 (54.9%)	
Female	21 (47.7%)	23 (45.1%)	
ASI (mm)			0.8370
Mean	18.2	17.7	
SD	12.1	11.5	
Largest stone size (mm)			0.6445[@]
Mean	6.3	6.7	
SD	4.2	4.2	
Stone number			1.0*
Median	4	4	
Minimum	1	1	
Maximum	8	8	

[@]Unpaired t-test; [#]Chi-square test; *Wilcoxon rank sum test

ASI: aggregate stone index; SD: standard deviation

Table 3 Melamine concentration in the milk consumed by Sichuan and Hong Kong children

	Sichuan children (n=44)*	Melamine concentration
三鹿 (Sanlu)	31	>5500 mg/kg[#]
南山 (Nanshan)	3	>5500 mg/kg[#]
雅士利 (Yashili)	3	53.4 mg/kg[#]
聖元 (Synutra)	2	150 mg/kg[#]
施恩 (Scient)	4	17.0 mg/kg[#]
伊利 (Yili)	1	8 mg/kg[#]
雀巢 (Nestle)	1	1.4 mg/kg[#]
多美滋 (Dumex)	1	0
Others	3	–

*5 patients consumed more than one type of milk

[#]Information from General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China

	Hong Kong children (n=22)*	Melamine concentration
蒙牛(Mengniu)	16	0[+]
伊利 (Yili)	11	5.5 mg/kg[+]
雀巢 (Nestle)	3	1.4 mg/kg[+]
維記 (Kowloon Dairy)	1	0
美贊臣 (Mead Johnson)	1	0
光明牌 (Bright Brand)	1	8.6 mg/kg[#]

*11 patients consumed more than one type of milk

[+]Centre for Food Safety, The Government of the Hong Kong, SAR

[#]Information from General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China

Significant difference in the melamine concentration of the milk consumed by the Sichuan and Hong Kong children ($p<0.0001$) by unpaired t-test.

Table 4 Melamine intake of the 15 Hong Kong children with MTMP consumption

Age (yr)	BW (Kg)	MTMP	Duration	Volume (ml)/day	Melamine (mg)/ BW (Kg)/day
10	36	伊利 (Yili)	5 yr	750-1000	0.115-0.153
10	34.5	伊利 (Yili)	2 yr	500	0.080
3	18.9	伊利 (Yili)	1.5 yr	1000	0.291
12	29.5	伊利 (Yili)	6 yr	500	0.093
8	22.8	伊利 (Yili)	6 yr	250-500	0.060-0.121
12	50.4	伊利 (Yili)	4 yr	500	0.055
13	41.2	伊利 (Yili)	5 yr	250-500	0.033-0.067
1	12.6	伊利 (Yili)	4 mo	250	0.109
2	14.2	伊利 (Yili)	14 mo	250-500	0.097-0.194
11	43.4	伊利 (Yili)	3 yr	250-500	0.032-0.063
7	22	伊利 (Yili)	5 yr	250-500	0.063-0.125
4	17	雀巢 (Nestle)	4 yr	1250	0.103
4	15	雀巢 (Nestle)	3 yr	250-480	0.023-0.045
4	15.7	雀巢 (Nestle)	4 yr	480	0.043
4	18	光明牌 (Bright Brand)	1 yr	300	0.143

BW: body weight; yr: year; mo: month; MTMP: melamine-tainted milk product

Table 5 Characteristics of stone at diagnosis between children with their stones passed out and not passed out completely

Sichuan children			
	Pass out n=31	**Not pass out / lost follow-up n=13**	**Wilcoxon rank p-value**
Stone number	4.9±3.0	2.5±2.1	**0.0139**
Largest stone size	5.1±3.6	9.2±3.9	**0.002**
Hong Kong children			
	Pass out n=13	**Not pass out / lost follow-up n=9**	**Wilcoxon rank p-value**
Stone number	1.3±0.48	1.22 ±0.44	0.6972
Largest stone size	3.6±1.6	4.1±1.6	0.4606

Figure 2 shows the time at which all the renal stones passed out was negatively correlated to the number of stones at diagnosis in Sichuan children (r=-0.47, p=0.008) but no such correlation in Hong Kong children (r=-0.026, p=0.93).

Figure 3 shows the urinary IL-8/creatinine and MCP-1/creatinine ratios in Sichuan and Hong Kong children at 6, 9, 12 months follow-up. For Hong Kong children, the analysis was done for the whole cohort of 22 (Figure 3a) and the 15 who had MTMP consumption (Figure 3b). At 6 and 9 months follow-up, Sichuan children with renal stones had significantly higher urinary IL-8/creatinine ratio than other groups, suggesting melamine stones could induce renal interstitial inflammation. Urinary IL-8/creatinine ratio in Sichuan children with renal stones declined from 6-month to 12-month follow-up, reaching levels similar to those with renal stones discharged completely, suggesting the inflammation could largely subside despite of the persistence of melamine stones. For Hong Kong children, the urinary IL-8/creatinine ratio was similar for those with or without renal stones. Moreover, the urinary IL-8/creatinine ratios of these 2 groups of Hong Kong children were similar to those of Sichuan children with stones discharged completely and lower than that in Sichuan children with persisting renal stones (Figure 3). Moreover, there were no difference in the urinary IL-8/creatinine and MCP-1/creatinine ratios at 6, 9 and 12 months between the Hong Kong children with and without MTMP consumption (Table 6).

The urinary MCP-1/creatinine ratios only showed marginal significance at 6 months follow-up for the ANOVA analysis, but not for the post hoc test (Figure 3).

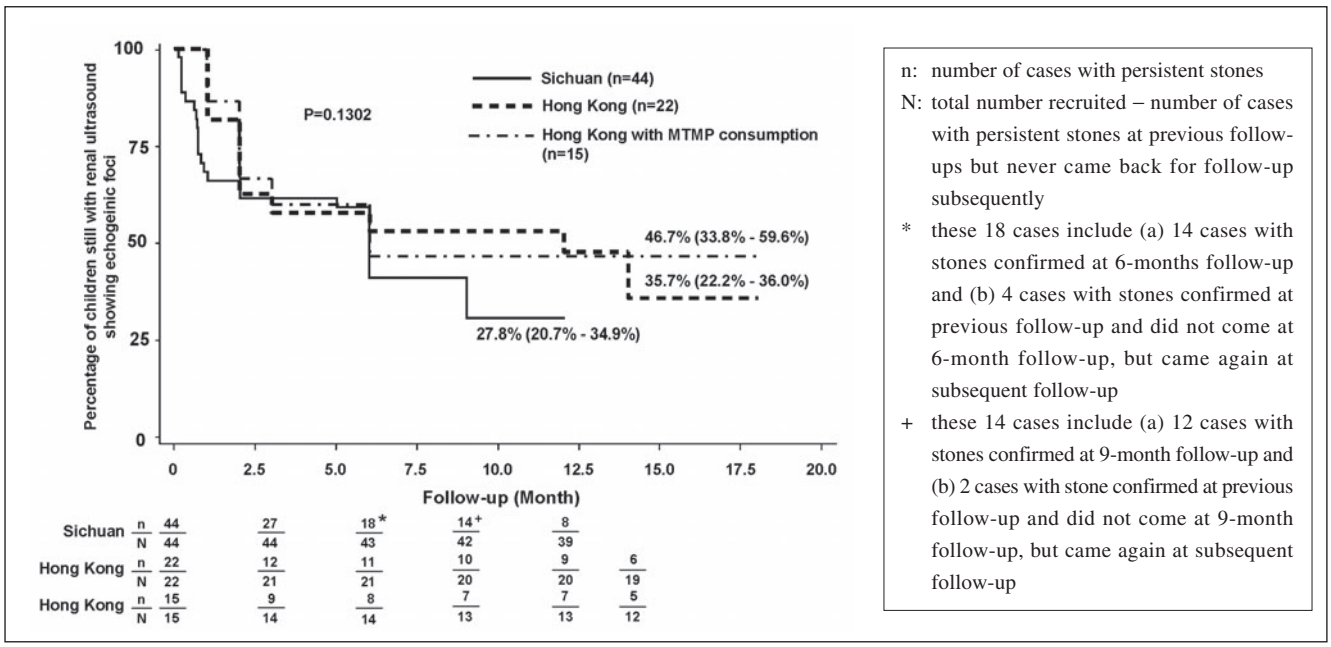

Figure 1 The Kaplan-Meier curve of the presence of renal stones in the 44 Sichuan children, the 22 Hong Kong children screened positive by SAC with ultrasound and the 15 Hong Kong children with MTMP consumption.

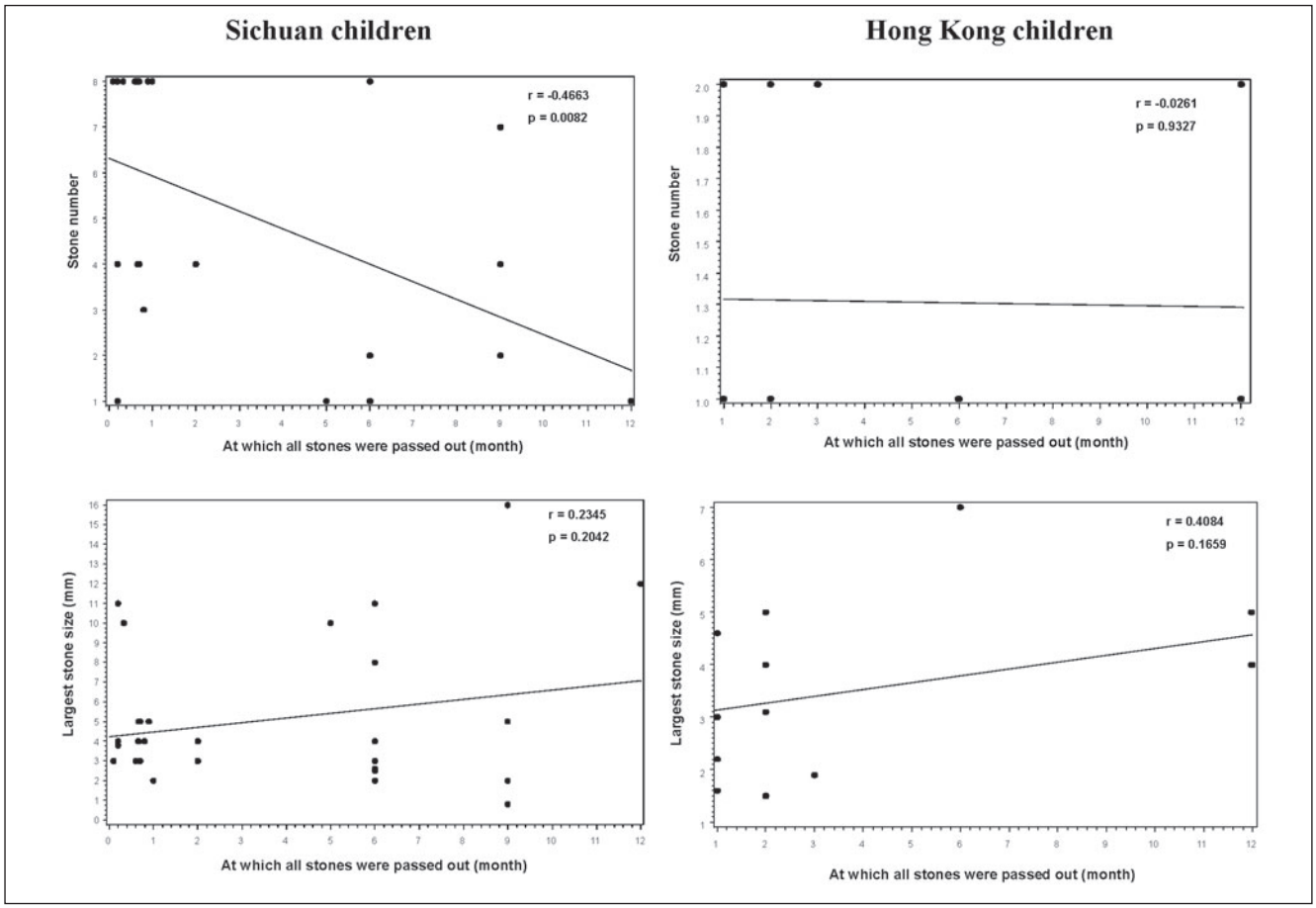

Figure 2 Relation of stone number and size with the time when they were passed out completely.

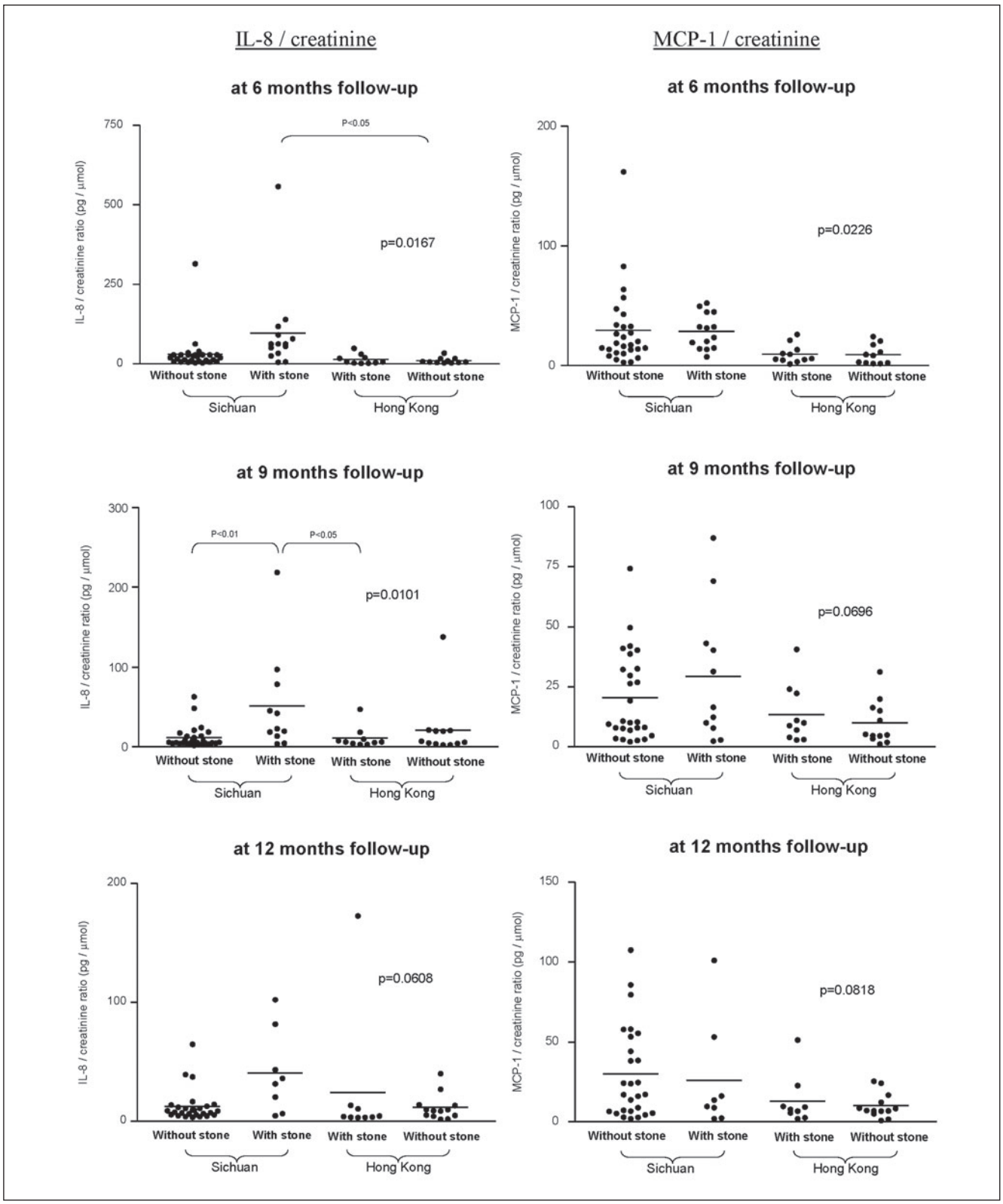

Figure 3a Urinary IL-8/creatinine and MCP-1/creatinine ratios in the 44 Sichuan and the 22 Hong Kong children screened positive by SAC with ultrasound.

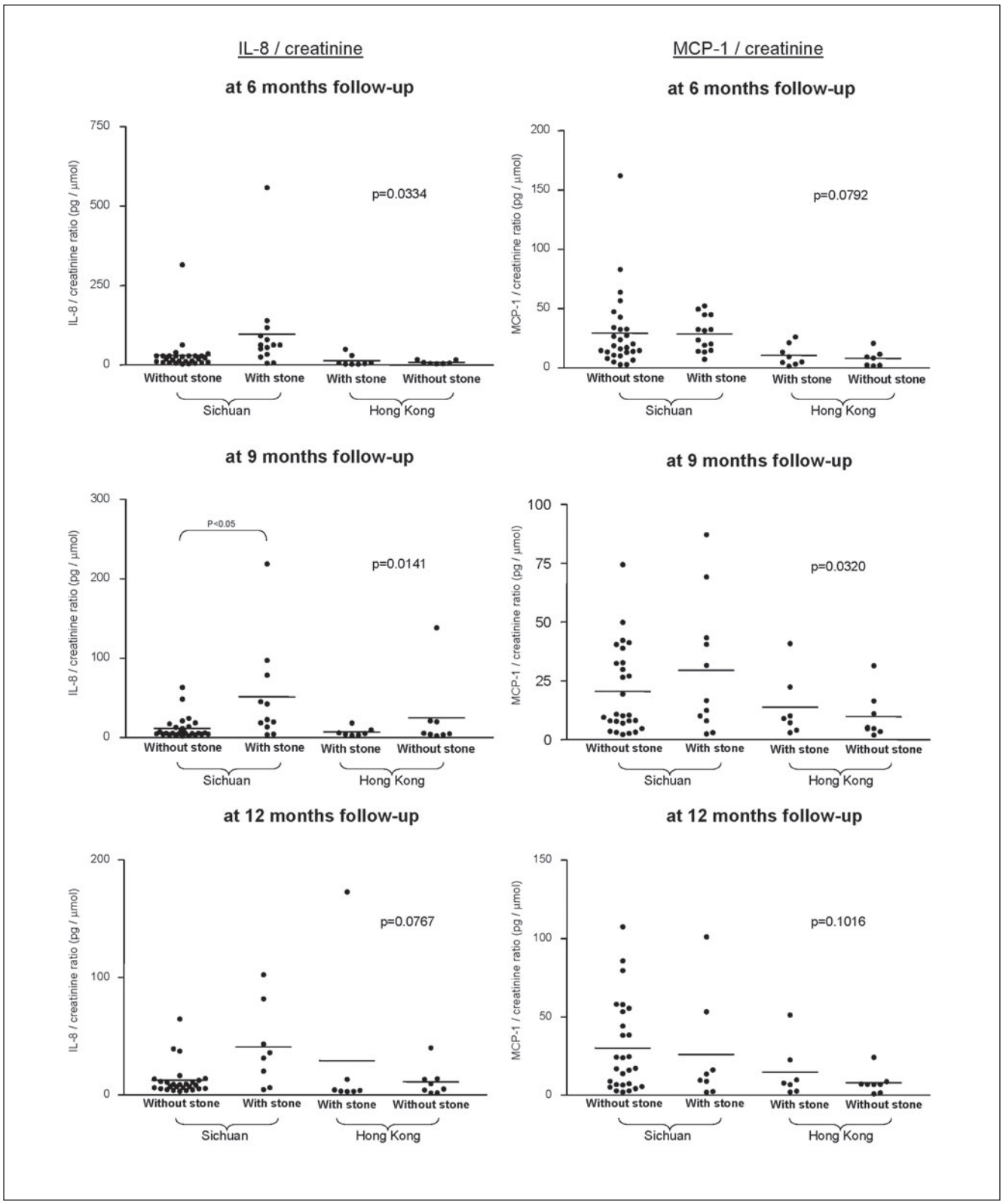

Figure 3b Urinary IL-8/creatinine and MCP-1/creatinine ratios in the 44 Sichuan and the 15 Hong Kong children with MTMP consumption.

Table 6 No difference in urinary IL-8/creatinine and MCP-1/creatinine ratios between the Hong Kong children with and without MTMP consumption

	No MTMP (n=7)	MTMP (n=15)	p-value
6 months			
MCP-1 / Urine creatinine ratio (pg/μmol)			0.7972[@]
Mean	10.4	9.4	
SD	8.8	7.9	
IL-8 / Urine creatinine ratio (pg/μmol)			0.5073[@]
Mean	15.0	10.9	
SD	11.3	13.0	
9 months			
MCP-1 / Urine creatinine ratio (pg/μmol)			0.9538[@]
Mean	11.4	11.7	
SD	8.8	11.5	
IL-8 / Urine creatinine ratio (pg/μmol)			0.9478[@]
Mean	15.7	16.6	
SD	15.5	34.2	
12 months			
MCP-1 / Urine creatinine ratio (pg/μmol)			0.8825[@]
Mean	11.9	11.1	
SD	7.2	13.0	
IL-8 / Urine creatinine ratio (pg/μmol)			0.5928[@]
Mean	10.5	19.6	
SD	7.7	43.4	

[@]Unpaired t-test

IL8: interleukin 8; MCP-1: monocyte chemoattractant protein 1; MTMP: melamine-tainted milk product; SD: standard deviation

Discussion

Our hypothesis that Hong Kong children screened positive to have renal stones with ultrasound by the Special Assessment Centres (SAC) did not have melamine associated renal stones is supported by the findings in the present study. First and foremost the melamine concentration in the milk consumed was much lower in the Hong Kong children as compared to the Sichuan children (Table 3). Moreover, some of the Hong Kong children did not even consume MTMP. Evidence reported from other mainland Chinese studies also seems to substantiate our hypothesis and will be discussed as follows.

The prevalence of renal stones or echogenic foci identified by ultrasound in Hong Kong has been reported to range from 0.03% to 0.6%[14-16] with our present study showing a prevalence rate of 0.205%. These figures are much lower than that in Chongqing (2.51%),[17] Hangzhou (3.61%),[18] Sichuan (2.58%)[3] and Beijing (2.9%).[2] Indeed the prevalence of renal stones among children not exposed to MTMP in Chongqing was estimated to be 0.41%,[17] not much different from the prevalence reported in Hong Kong, suggesting the so-called melamine associated renal stones in Hong Kong could represent largely background rate of renal stones not related to melamine toxicity. Even if some of these stones were related to melamine, these were results from relatively low level of melamine exposure because firstly Hong Kong patients were much older than Sichuan patients (Table 1), hence would consume MTMP at a much lower proportion of total dietary intake, and secondly the highest melamine concentration of mainland Chinese MTMP was >5500 mg/Kg[3] while that for Hong Kong MTMP was much lower[19,20] (Table 3). Indeed the melamine intakes of the 15 Hong Kong children with MTMP consumption were below the tolerable daily intake of 0.2 mg melamine/Kg body weight as stipulated by the World Health Organisation[3] except one, who consumed 0.29 mg/Kg/day (Table 4).

The number of renal stones and the largest renal stone size were significantly higher in Sichuan children than in Hong Kong children (Table 1), either because of the difference in the level of melamine exposure or the findings in Hong Kong children were largely not related to melamine exposure but due to other unknown causes.

The resolution rates of the renal stones at 1-year follow-up were lower in both the Sichuan and Hong Kong children (72% and 52%) in our present study as compared to that in Beijing (95.5%).[4] The Sichuan children were likely to suffer from more severe renal disease as they were inpatients with larger number of stones of larger size as compared to the outpatients.[3] Indeed we have demonstrated in our previous study children with larger stone size could not pass out their renal stones as quickly as those with smaller stone size.[3] In our present study, the Sichuan children who passed out all the stones had significantly larger number of stones and smaller stones but no such relationship for Hong Kong children (Table 5). The Beijing patients[4] suffered from less severe renal disease as compared to our Sichuan cohort,[3] hence it is not surprising the Beijing children had a higher resolution rate than our Sichuan children. As for the Hong Kong children with even lower resolution rate of their renal stones, the likely explanation is that their stones were not related to melamine, hence no valid comparison can be made. It is to be emphasized again that Hong Kong children have renal stones of smaller size as compared to Sichuan children (Table 1), hence if they have melamine-associated renal stones, they should be able to discharge them much

faster according to the evidence from our previous study.[3]

Inflammatory cytokines IL-8 and MCP-1 play pivotal roles in the physiologic and pathologic processes during inflammation.[12] IL-8 and MCP-1 are involved in the migration and localisation of the leukocytes to the inflammatory or injury sites during renal inflammatory injury. [21] Renal epithelial cells exposed to uric acid crystals synthesize MCP-1.[12,21] Monocytes or neutrophils exposed to urate crystals produce IL-8.[12,22-24] Therefore, urinary IL-8 and MCP-1 could be markers for renal inflammation. Urinary IL-8/creatinine ratio was increased in Sichuan children with melamine associated renal stones as compared to those who discharged their stones completely at 9 months follow up (Figure 3). Urinary IL-8/creatinine ratio declined to low levels in most Sichuan children once the renal stones were completely discharged, suggesting urinary IL-8/creatinine ratio could be useful to monitor renal inflammation. Even with persisting renal stones, the urinary IL-8/creatinine ratio in Sichuan children declined over the 1-year follow-up, suggesting partial resolution of inflammation may occur even in the presence of melamine-associated renal stones. Such an elevation and change of urinary IL-8/creatinine ratio was not seen in Hong Kong children (Figure 3). Moreover there were no difference in the urinary IL-8/creatinine ratios between Hong Kong children with and without MTMP consumption (Table 6). Urinary MCP-1/creatinine ratio does not seem to be useful for renal inflammation associated with melamine stones.

Our present study is limited in several aspects, including the fact that the Sichuan cohort was recruited from inpatients, hence not representative of the more general population of children with melamine-associated renal stones. This would also compromise to some extent the validity of direct comparison of these Sichuan children with Hong Kong children who were largely asymptomatic and screened positive with echogenic foci by renal ultrasound at community level. Nevertheless, the Sichuan outpatient children with melamine-associated renal stones still have much higher number of larger stone size[3] than that of our Hong Kong children, further suggesting the renal stones in Hong Kong children may not be melamine related. The most important piece of evidence that the renal stones in our Hong Kong children may not be related to melamine is that the melamine concentration of the milk consumed was either zero or much lower than that consumed by Sichuan children, resulting in only one Hong Kong child having consumed more than the tolerable daily intake of melamine. Difficulty in following up the Sichuan children was also encountered in the present study because some of them lived far away from Chengdu, rendering the 1-year follow-up not complete for the 44 children. Using Kaplan-Meier analysis has to some extent resolved this issue.

To conclude, about 28% of Sichuan children with melamine-associated renal stones still had the renal stones at 12-month follow-up with some evidence of renal inflammation. This figure is likely to be an over-estimate for the general population of children with melamine-associated renal stones as our Sichuan cohort was recruited from the more severe inpatient population. Whereas 48% of Hong Kong children suspected to have melamine-associated renal stones still had the renal stones at 12-month follow-up, such high persistence rate is not compatible with these renal stones being melamine related. Taking into account of all the evidences above, Hong Kong children screened positive to have renal stones with ultrasound by the SAC most likely did not suffer from melamine-associated renal stones.

Acknowledgements

This work was supported in part by the Hong Kong SAR Government (Research Fund for Studies Related to Melamine Incident MI-FU-08).

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