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Case-control study of Sichuan and Hong Kong children with melamine-associated renal stones: renal ultrasonography and urinary IL-8 and MCP-1 levels

Key Messages

1. The clinical features of Sichuan and Hong Kong children with renal stones differ significantly, suggesting different aetiological pathways.
2. Urinary interleukin-8/creatinine ratio was higher in children having renal stones in Sichuan, but in Hong Kong it was similar in children with or without renal stones. This suggests different inflammatory potentials.
3. A large percentage of children still had renal stones at the 1-year follow-up.

背景：2008年，中國兒童食用受三聚氰胺污染奶粉後有腎結石症狀。檢查的22 384 000名兒童中有294 000名被診斷患有三聚氰胺相關腎結石，其中51 900名全國公開報導。我們曾調查過189名患腎結石的四川患兒，發現此種情況在嬰兒更常見，來自貧困家庭的患兒病情更嚴重。這些患兒的遠期情況尚不清楚。另外，同時在香港，轉介的27 000名兒童經過腎臟超聲檢查後，有15名懷疑患有結石並上報衛生防護中心。這些腎結石的性質仍不清楚。

目的：研究假設四川患兒三聚氰胺暴露量大，患有真正三聚氰胺相關腎結石；但是香港兒童三聚氰胺暴露量小，臨床特徵可能不同，所患腎結石可能與三聚氰胺無關。在隨訪的一年中，我們比較這兩組兒童的腎臟超聲結果及尿液中炎性細胞因子的水平。

研究設計和方法：在前次研究中，四川華西第二教學醫院確診了51名三聚氰胺腎結石患兒。本次研究中納入44位作為三聚氰胺腎結石真正病例。香港的六間醫院利用腎臟超聲篩查了16 567名12歲以下的兒童，有34名被懷疑患有三聚氰胺腎結石，我們納入其中22名作為本研究的對照組。在隨訪的第6、9以及12個月，對腎臟超聲和尿液中IL-8和MCP-1水平進行了比較。

結果：香港兒童的年齡（ 75.0 ± 42.1 月）明顯比四川兒童（ 25.7 ± 23.8 月）大。四川兒童腎結石數量（1-8，中位數為4）顯著高於香港兒童（1-2，中位數為1）。四川兒童最大結石的尺寸（ 6.3 ± 4.2 毫米）顯著大於香港兒童（ 3.8 ± 1.6 毫米）。隨訪一年後，28%的四川兒童和48%的香港兒童仍有腎結石。在第6個月，仍有腎結石的四川患兒的尿液IL-8水平顯著高於結石完全排出的四川患兒，以及高於已排出或未排出結石的香港兒童（ $P < 0.0001$ ）。香港有或沒有腎結石的兒童之間尿液IL-8水平相似。尿液IL-8水平與最大結石的尺寸在四川兒童中具有顯著相關性（ $r = 0.82$, $P < 0.0001$ ），但在香港兒童中無相關性（ $r = 0.21$, $P = 0.36$ ）。在各組兒童之間，尿液MCP-1水平無明顯差異。

結論：四川和香港腎結石兒童的臨床特徵顯著不同，顯示其病因來源不同。尿液IL-8水平在四川腎結石患兒中較高，但在香港有或沒有腎結石的兒童中相似，顯示其具有不同的致炎性。

意義：在隨訪的一年中，有相當部分兒童仍患有腎結石。香港兒童的腎結石與四川患兒的腎結石在本質上不同。有必要進行長期隨訪。

Introduction

In September 2008, there was an outbreak of melamine-associated renal stones in children in China.¹ Melamine was added to infant formula to elevate protein content. According to the Chinese Ministry of Health, in December 2008, 22 384 000 children with suspected melamine exposure were examined. Of whom, 294 000 were diagnosed as having urinary stones and 51 900 were hospitalised. In our previous study, 189 (2.6%) of 7328 children who presented to the West China Second University Hospital in Sichuan with melamine exposure were identified by ultrasonography as having urinary stones.² Melamine-associated urinary stones occurred more frequently in infants, and children from poorer families were more seriously affected.

In response, the Hong Kong SAR Government formed an ad hoc expert group to offer advices on the clinical care of suspected cases, the public health response, research, and food safety. According to the report submitted on 15 April 2009, 56 847 children were screened in designated clinics. Of these, 27 616 were

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Table 1. Kidney stone size and number at presentation in Sichuan and Hong Kong patients

Parameter	Sichuan patients (n=44)	Hong Kong patients (n=22)	P value (test)
Mean±SD age (months)	25.7±23.8	75.0±42.1	<0.0001 (Unpaired t-test)
Sex			0.542 (Chi-square test)
No. (%) of males	23 (52.3)	9 (40.9)	
No. (%) of females	21 (47.7)	13 (59.1)	
Mean±SD aggregate stone index (mm)	18.2±12.1	4.3±1.7	<0.0001 (Unpaired t-test)
Mean±SD largest stone size (mm)	6.3±4.2	3.8±1.6	0.0087 (Unpaired t-test)
Median (range) stone number	4 (1-8)	1 (1-2)	<0.0001 (Wilcoxon rank sum test)

Table 2. Melamine concentration in milk consumed by Sichuan and Hong Kong patients

Brand	Patients*	Melamine concentration† (mg/kg)
	Sichuan (n=44)	
三鹿 (Sanlu)	31	>5500
南山 (Nanshan)	3	>5500
雅士利 (Yashili)	3	53.4
圣元 (Synutra)	2	150
施恩 (Scient)	4	17.0
伊利 (Yili)	1	8
雀巢 (Nestle)	1	1.4
多美滋 (Dumex)	1	0
Others	3	-
	Hong Kong (n=22)	
蒙牛 (Mengniu)	16	0
伊利 (Yili)	11	5.5
雀巢 (Nestle)	3	1.4
維記 (Kowloon Dairy)	1	0
美贊臣 (Mead Johnson)	1	0
光明牌 (Bright Brand)	1	8.6

* Five Sichuan and 11 Hong Kong 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, or Centre for Food Safety, The Government of the Hong Kong SAR. The melamine concentration of the milk consumed by the Sichuan and Hong Kong children was significantly different ($P<0.0001$, unpaired *t*-test).

referred to Special Assessment Centres where further tests including renal ultrasonography were performed.

Our hypothesis was that Sichuan children with heavy melamine exposure suffered from genuine melamine-associated renal stones, whereas Hong Kong children with minimal melamine exposure had different clinical features, so that their renal stones may not be melamine-related. This study aimed to compare the clinical features and the renal ultrasound findings, as well as the urinary inflammatory cytokine/creatinine ratios in the Sichuan and Hong Kong children at the 1-year follow-up.

Methods

This study was conducted from April 2009 to September 2010. In our previous study, 44 out of 51 Sichuan children admitted to the West China Second University Hospital with suspected melamine-associated renal stones were identified as genuine cases.² In Hong Kong, 16 567 children under

12 years of age underwent renal ultrasonography in Kwong Wah Hospital, Pamela Youde Nethersole Eastern Hospital, Queen Elizabeth Hospital, Queen Mary Hospital, Tuen Mun Hospital, and United Christian Hospital. Of them, 22 of 34 children suspected to have melamine-associated renal stones were recruited as controls. All these cases and controls were followed up using renal ultrasonography and urinary IL-8 and monocyte chemotactic protein-1 (MCP-1) at about 6, 9, and 12 months.

Results

The Sichuan children were significantly younger than the Hong Kong children ($P<0.0001$, Table 1), but the gender distribution was similar. The largest stone size and the number of stones at presentation were significantly greater in the Sichuan than Hong Kong children ($P=0.0087$ and $P<0.0001$, respectively). The melamine concentration in the milk consumed by Sichuan children was significantly higher ($P<0.0001$, Table 2).

Figure 1 shows the Kaplan-Meier plot of the presence of renal stones in Sichuan and Hong Kong children. At the 12-month follow-up, about 28% of Sichuan children and 48% of Hong Kong children still had evidence of renal stones ($P=0.1302$). In two Sichuan children, their renal stones were discharged, but they still had hydronephrosis at the 9- and 12-month follow-up. One Hong Kong child who had a linear echogenic focus with double-line configuration (suggestive of vascular calcification rather than renal stone) was classified as having a stone.

Figure 2 shows the urinary IL-8/creatinine and MCP-1/creatinine ratios in Sichuan and Hong Kong children at the 6, 9, 12 months follow-up. At months 6 and 9, Sichuan children with renal stones had significantly higher urinary IL-8/creatinine ratio. This suggested that melamine stones could induce renal interstitial inflammation. The urinary IL-8/creatinine ratio in Sichuan children with renal stones declined from month 6 to month 12, reaching levels similar to those whose renal stones was discharged completely. This indicated that such inflammation could largely subside, despite of the persistence of melamine stones. In Hong Kong children, the urinary IL-8/creatinine ratio was similar to that in subjects with or without renal stones. Moreover, the ratios in these two groups of Hong Kong

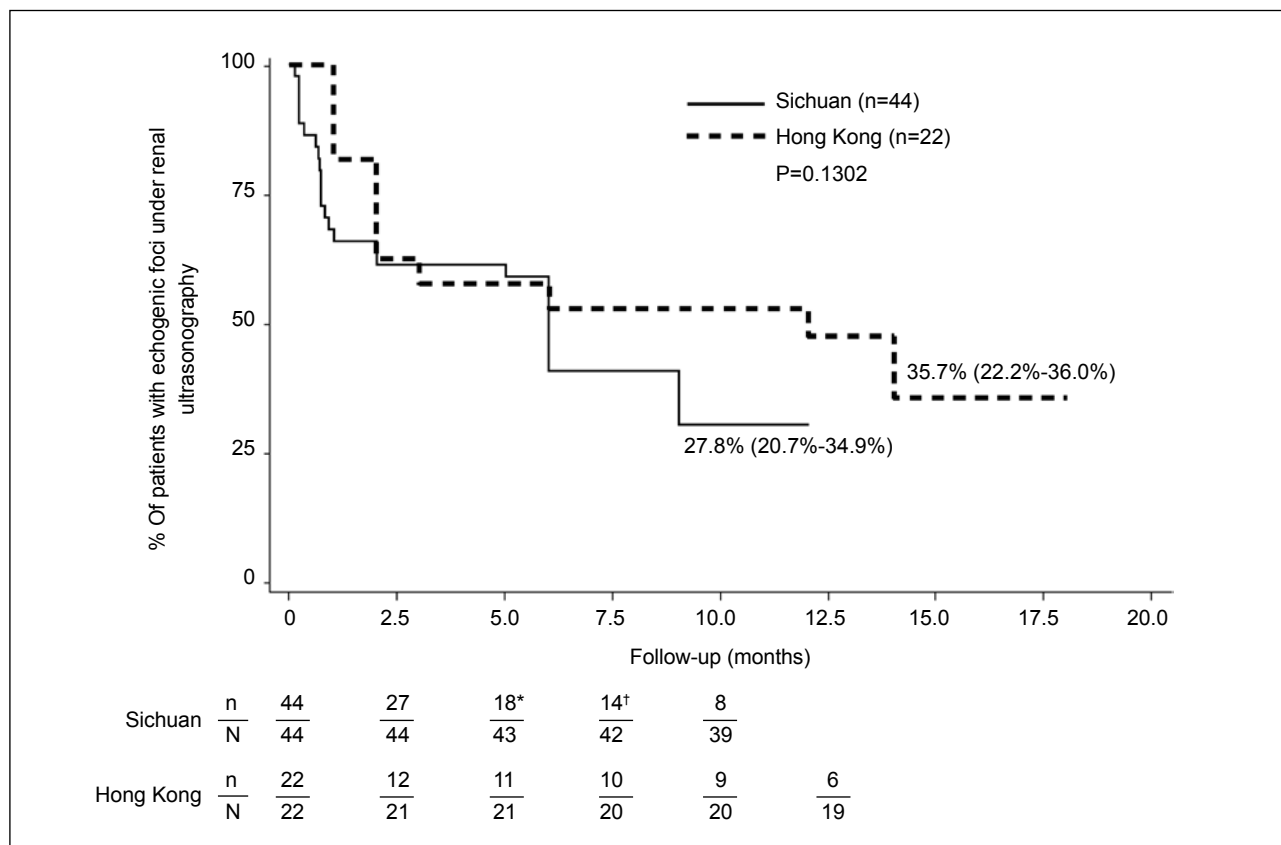


Fig 1. The Kaplan-Meier plot of the presence of renal stones in Sichuan and Hong Kong patients

* Including 14 cases with stones confirmed at 6-month follow-up and four cases with stones confirmed at previous follow-up and did not come at 6-month follow-up, but came again at subsequent follow-up
 † Including 12 cases with stones confirmed at 9-month follow-up and two cases with stone confirmed at previous follow-up and did not come at 9-month follow-up, but came again at subsequent follow-up

children were similar to those of Sichuan children whose stones were discharged completely and were lower than in Sichuan children with persisting renal stones (Fig 2).

The urinary MCP-1/creatinine ratios only showed marginal significance at the 6-month follow-up in the ANOVA analysis, and not at all in the post hoc test (Fig 2).

Discussion

In Hong Kong children, the prevalence of renal stones or echogenic foci identified by ultrasonography has been reported be 0.03% to 0.6%.³ In the present study it was 0.205%. These figures are much lower than those in Chongqing (2.51%),⁴ Hangzhou (3.61%), Sichuan (2.58%), and Beijing (2.9%). In fact, among children not exposed to melamine-tainted milk products (MTMP) in Chongqing, the prevalence was about 0.41%.⁴ This suggests that Hong Kong children with ‘melamine-associated’ renal stones had largely incidental findings not related to melamine toxicity. Even if some of these stones were related to melamine, the levels of exposure were relatively low, because Hong Kong patients were much older than Sichuan patients, and hence consumed much lower quantities of MTMP per kg body

weight. In addition, the highest melamine concentration in mainland Chinese MTMP was >5500 mg/kg,³ whereas it was much lower in Hong Kong.

The number of renal stones and the largest renal stones were significantly greater in Sichuan children than in Hong Kong children. This was presumably due to differences in the levels of melamine exposure.

The resolution rates of the renal stones at the 1-year follow-up were low in both the Sichuan and Hong Kong children (72% and 52%, respectively), compared to Beijing children (95.5%).⁵ The Sichuan patients probably had more severe renal disease because of the greater number and size of stones.² Larger stones could not be passed out as readily as smaller stones.² Beijing patients⁵ had less severe renal disease than the Sichuan cohort.² Hong Kong children had even lower resolution rate for renal stones, probably because the stones were not truly related to melamine, even they were smaller.²

The urinary IL-8/creatinine ratio is a useful marker for kidney inflammation secondary to melamine-associated renal stones. The absence of elevated ratios in Hong

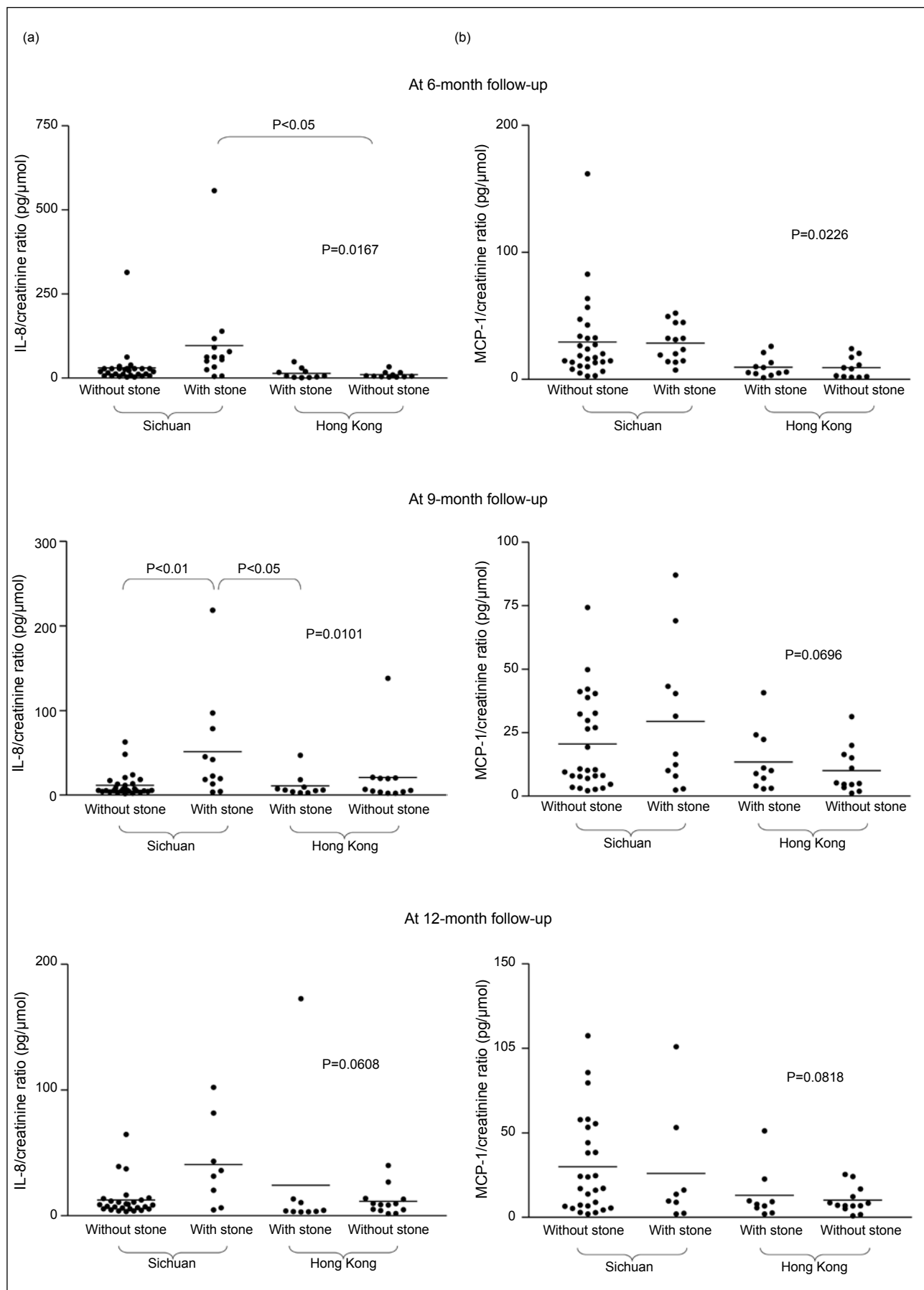


Fig 2. (a) Urinary IL-8/creatinine and (b) MCP-1/creatinine ratios in Sichuan and Hong Kong patients with or without renal stones over 1-year follow-up

Kong children with renal stones suggests that the stones may not have been truly related to melamine or that the stones were too small to cause IL-8 induction. The ratio decreased in most Sichuan children once the stones were completely discharged. This suggests that the ratio can be a means of monitoring renal interstitial inflammation. Even with persisting renal stones, the ratio in Sichuan children decreased over 1 year, suggesting that inflammation may resolve partially even if the stones persist. The ratio did not seem to be useful for monitoring renal inflammation.

Limitations of the present study were that the Sichuan cohort was recruited from inpatients, hence not representative of the general population with melamine-associated renal stones. This compromised the validity of direct comparison of clinically ill Sichuan children with Hong Kong children who were largely asymptomatic. The melamine-associated renal stones were more and larger in Sichuan outpatient children than in Hong Kong children.² This suggests that the renal stones in Hong Kong children may not be melamine related. In addition, the melamine concentration of the milk consumed by Hong Kong children was much lower than that consumed by Sichuan children. Some Sichuan children lived far from Chengdu, and 44 of them did not complete the 1-year follow-up. The use of the Kaplan-Meier analysis resolved this issue in some extent.

Conclusion

At the 1-year follow-up, about 28% of Sichuan children with melamine-associated renal stones still had renal stones and some evidence of renal interstitial inflammation. This figure is likely to be an overestimate for the general population of such children, as our Sichuan cohort was recruited from a more severely affected inpatient population. There was little evidence of renal interstitial inflammation in Hong

Kong children with and without renal stones.

Mainland Chinese children with persistent melamine-associated renal stones should have long-term follow-up to monitor possible renal interstitial inflammation and fibrosis. For Hong Kong children with suspected 'melamine-associated' renal stones, the stones were unlikely to be related to melamine and no significant clinical harm was likely to ensue.

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