

# Cadmium-Exposed Population in Mae Sot District, Tak Province: 3. Associations between Urinary Cadmium and Renal Dysfunction, Hypertension, Diabetes, and Urinary Stones

Witaya Swaddiwudhipong MD\*, Pisit Limpatanachote MD\*\*,  
Muneko Nishijo MD, PhD\*\*\*, Ryumon Honda PhD\*\*\*\*,  
Pranee Mahasakpan BSc, MPH\*, Somyot Krintratun MD\*\*

\* Department of Community and Social Medicine, Mae Sot General Hospital, Tak

\*\* Department of Internal Medicine, Mae Sot General Hospital, Tak

\*\*\* Department of Public Health, Kanazawa Medical University, Japan

\*\*\*\* Department of Social and Environmental Health, Kanazawa Medical University, Japan

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**Objective:** To study the associations between urinary cadmium and renal dysfunction, hypertension, diabetes, and urinary stones in an adult population living in cadmium-contaminated areas in Mae Sot District, Tak Province, Thailand.

**Material and Method:** Seven hundred ninety five cadmium-exposed adults were screened for urinary cadmium, renal dysfunction, hypertension, diabetes, and urinary stones in 2005. Six selected markers of renal function in the present study were urinary excretion of  $\beta_2$ -microglobulin ( $\beta_2$ -MG), N-acetyl- $\beta$ -D-glucosaminidase (NAG), total protein and calcium, serum creatinine, and glomerular filtration rate (GFR).

**Results:** The mean age of the study persons was 50 years old. The overall prevalence rates of hypertension, diabetes, and urinary stones were 33.3%, 6.2%, and 8.9% respectively. The prevalence of increased proteinuria was greatest in those with urinary cadmium levels  $\geq 15$   $\mu$ g/g creatinine. Urinary excretion of  $\beta_2$ -MG, NAG, and total protein significantly increased with increasing urinary cadmium levels, after adjusting for other co-variables by multiple linear regression analysis. However, urinary cadmium was not significantly associated with urinary calcium, serum creatinine, and GFR. The prevalence rates of hypertension, diabetes, and urinary stones did not significantly increase with increasing urinary cadmium levels. Hypertension, diabetes, and urinary stones were also significant predictors of impaired renal function.

**Conclusion:** In this population, increasing levels of urinary cadmium are associated with increasing urinary excretion of  $\beta_2$ -MG, NAG, and total protein. Risk for hypertension, diabetes, and urinary stones remains uncertain in relation to cadmium exposure.

**Keywords:** Cadmium, Renal dysfunction, Hypertension, Diabetes, Urinary stone

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Cadmium levels in agricultural crops grown in cadmium-contaminated areas have been found to be elevated and can be many times above normal levels<sup>(1,2)</sup>. Long-term oral exposure may lead to chronic cadmium poisoning. Urinary excretion of cadmium is a useful

measure for prolonged cadmium exposure and body burden<sup>(1-7)</sup>. The kidney is considered the critical target organ for chronic cadmium exposure<sup>(1-8)</sup>. An initial sign of cadmium-induced nephrotoxicity is tubular proteinuria, usually demonstrated by increased urinary excretion of low molecular weight proteins such as  $\beta_2$ -microglobulin ( $\beta_2$ -MG), retinol binding protein,  $\alpha_1$ -microglobulin, enzymes such as N-acetyl- $\beta$ -D-glucosaminidase (NAG), and calcium. Prolonged

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Correspondence to: Swaddiwudhipong W, Department of Community and Social Medicine, Mae Sot General Hospital, Tak 63110, Thailand. Phone: 055-531-229, Fax: 055-533-046. E-mail: swaddi@hotmail.com

exposure to cadmium, tubular dysfunction may progress and lead to increased blood creatinine, decreased glomerular filtration rate (GFR), and end-stage renal disease<sup>(1-8)</sup>. However, risk for hypertension and diabetes is not conclusively a result of cadmium exposure<sup>(1-3,5)</sup>.

Cadmium is a widely but sparsely distributed element found in the earth's crust, primarily in association with zinc ores. Cadmium is a common by-product during the processing of zinc-bearing ores. In Mae Sot District, Tak Province, northwestern Thailand, paddy fields were irrigated from two creeks that passed through an area where a zinc mine had been actively operated for > 20 years. Crops, including rice, grown in the areas were found to contain markedly elevated cadmium levels during the surveys in 2001-2004<sup>(9-12)</sup>. The cadmium-contaminated areas were discovered in the 12 villages of the district. Since the majority of inhabitants consumed rice and other crops grown locally, they were at risk of cadmium-induced nephropathy. Of the 7,697 cadmium-exposed adults examined for urinary cadmium by the Mae Sot General Hospital in 2004, 554 (7.2%) had urinary cadmium levels  $\geq 5 \mu\text{g/g}$  creatinine<sup>(13)</sup>. Persons with high urinary cadmium levels ( $\geq 5 \mu\text{g/g}$  creatinine) were then screened for renal effects in 2004 and a significant number of them had renal dysfunction<sup>(14)</sup>. They were reexamined for urinary cadmium, renal dysfunction, hypertension, diabetes, and urinary stones in 2005. In order to determine the cadmium toxic effects among those with urinary cadmium levels  $< 5 \mu\text{g/g}$  creatinine, the authors randomly selected a sample of about 300 adults who lived in the contaminated areas and contained urinary cadmium levels  $< 5 \mu\text{g/g}$  creatinine for similar examinations. The present study compared the prevalence of renal dysfunction, hypertension, diabetes, and urinary stones among these study persons categorized by level of urinary cadmium. The present study also determined the associations between urinary cadmium and renal dysfunction, hypertension, diabetes, and urinary stones.

## Material and Method

### Study population

The present study included those 554 persons with urinary cadmium levels  $\geq 5 \mu\text{g/g}$  creatinine during the 2004 survey and 300 (20-30 per village according to their sizes) with urinary cadmium levels  $< 5 \mu\text{g/g}$  creatinine who were randomly selected by cluster sampling technique from these 12 cadmium-contaminated villages. Seven hundred ninety five (93.1%) of

the 854 persons participated in the present study. The participants were screened for urinary cadmium, renal dysfunction, hypertension, diabetes, and urinary stones in 2005. Six selected markers of renal function in the present study were urinary excretion of  $\beta_2$ -MG, NAG, total protein and calcium, serum creatinine, and GFR. The present study protocol was approved by the Hospital Ethical Committee and oral informed consent was obtained.

### Survey

The participants were interviewed about demographic characteristics, smoking status, and medical history of hypertension, diabetes, urinary stones, and renal diseases by trained health workers. Height and weight were measured to obtain body mass index (BMI,  $\text{kg/m}^2$ ). Blood pressure was measured twice on the right arm in a sitting position and the average was recorded. Hypertension was defined as diastolic pressure  $\geq 90$  mmHg and/or systolic pressure  $\geq 140$  mmHg or receipt of current antihypertensive medication.

Fasting venous blood was collected from each participant and forwarded to the laboratory of Mae Sot General Hospital within 2 hours of collection for analysis of plasma glucose by the enzymatic colorimetric method and serum creatinine by the Jaffe reaction method. The samples were measured by using the autoanalyzer (Konelab 30, Thermo Electron Corporation, Vantaa, Finland). The laboratory of the hospital has been certified for clinical chemistry analysis by the National External Quality Assessment Scheme in Clinical Chemistry, Thailand Ministry of Public Health, and by the External Quality Assessment in Clinical Chemistry, Faculty of Medical Technology, Mahidol University. Diabetes was defined as fasting plasma glucose  $\geq 126$  mg/dl on two occasions or currently receiving antidiabetic treatment. Estimation of GFR was made from the serum creatinine with the MDRD equation<sup>(15)</sup>.

A 30-ml sample of second morning urine was obtained from each participant. Two 3 ml aliquots from each urine sample were kept frozen ( $-20^\circ\text{C}$ ) for analysis of cadmium,  $\beta_2$ -MG, and NAG by the Department of Social and Environmental Health, Kanazawa Medical University, Japan. Prior to the storage, one drop of 0.5N sodium hydroxide was added to one of the two aliquots showing the pH of 5 or below to adjust the urine pH of 6-8 for prevention of further degradation of  $\beta_2$ -MG in an acid condition. The remaining urine samples were forwarded to the laboratory of Mae Sot

General Hospital for microscopic analysis and biochemistry measurements.

The urinary cadmium content was determined by a flameless atomic-absorption spectrometer (Shimadzu Model AA-6300, Japan). Urine standard reference material No. 2670 (The National Institute of Standards, USA) was used for quality control and validation of an analytical method. Urinary  $\beta_2$ -MG concentration was determined by a latex agglutination immunoassay (Eiken Chemical, Japan) and NAG content was determined by a colorimetric assay (Shionogi Pharmaceuticals, Japan). Urinary total protein and calcium concentrations were measured by the colorimetric method. Urinary creatinine concentration by the Jaffe reaction method was used to adjust for urinary excretion of cadmium and the study markers.

Participants who had a history of a urinary stone and/or hematuria were screened for the presence of a urinary stone by plain X-ray and ultrasonography. Prevalence of urinary stones was defined as presence of a urinary stone during the survey and/or reporting history of a urinary stone.

#### Statistical analysis

The distributions of variables were expressed in percentages of the present study persons categorized by level of urinary cadmium. The arithmetic mean and standard deviation were used to summarize the quantitative variables for each group of persons. The geometric mean was used when the logarithms of the observations (urinary  $\beta_2$ -MG, protein, and calcium) were more likely to distribute normally than the observations themselves. The chi-square test was used for comparison of proportions and analysis of variance or the Kruskal-Wallis test was used for comparison between means. Multiple linear or logistic regression analysis was used to determine the association between urinary cadmium and markers of renal function, hypertension, diabetes, and urinary stones.

#### Results

The characteristics of the 795 study persons are shown in Table 1. The mean age of the patients was 50 years old. The male to female ratio was 1:1.6. The proportion of current (daily and occasional) smokers was 44.9%. However, they were predominantly low-rate smokers: nearly all (98.6%) of daily smokers smoked < 10 cigarettes per day. The overall prevalence rates of hypertension, diabetes, and urinary stones were 33.3%, 6.2%, and 8.9% respectively.

Table 2 presents markers of renal dysfunction, prevalence of hypertension, diabetes, and urinary stones in the study persons categorized by level of urinary cadmium. The mean levels of urinary  $\beta_2$ -MG, NAG, and protein were highest in persons with urinary cadmium levels  $\geq 15 \mu\text{g/g}$  creatinine. There were no significant differences between the four groups in the prevalence rates of urinary calcium  $\geq 200 \text{ mg/g}$  creatinine, serum creatinine  $\geq 1.1 \text{ mg/dl}$ , and GFR  $< 60 \text{ ml/min/1.73 m}^2$ . Although the rates of hypertension, diabetes, and urinary stones were highest in persons with urinary cadmium levels  $\geq 15 \mu\text{g/g}$  creatinine, the differences between the four groups were not statistically significant.

Multiple linear regression analysis was used to determine the association between urinary cadmium and each of the six study renal markers separately, after adjusting for other co-variables. However, no study variables were found to correlate significantly with excretion of urinary calcium. Table 3 presents the results of the regression analysis of the determinants of the five study renal markers. The present study revealed that urinary excretion of  $\beta_2$ -MG, NAG, and protein significantly increased with increasing urinary cadmium levels, after adjusting for other co-variables.

**Table 1.** Characteristics, prevalence of hypertension, diabetes, and urinary stones in the 795 study persons, Mae Sot, Tak Province, Thailand

Variables	Distribution/mean
Age (years)	
Mean $\pm$ SD	50.2 $\pm$ 14.2
Sex	
Male (%)	38.2
Female (%)	61.8
Smoking status	
Never (%)	40.0
Former (%)	15.1
Current (%)	44.9
Body mass index ( $\text{kg/m}^2$ )	
Mean $\pm$ SD	22.0 $\pm$ 3.9
Hypertension	
Yes (%)	33.3
No (%)	66.7
Diabetes	
Yes (%)	6.2
No (%)	93.8
Urinary stone	
Yes (%)	8.9
No (%)	91.1

**Table 2.** Markers of renal dysfunction, prevalence of hypertension, diabetes, and urinary stones in the study persons by level of urinary cadmium

Renal markers	Urinary cadmium ( $\mu\text{g/g}$ creatinine)				p-value
	< 5	5-9.9	10-14.9	$\geq 15$	
Number of persons	311	314	97	73	
Urinary $\beta_2$ -MG ( $\mu\text{g/g}$ creatinine)					
$\geq 400$ (%)	20.6	32.2	26.8	42.5	<0.01
Mean $\pm$ SD*	136.0 $\pm$ 6.3	225.7 $\pm$ 6.8	171.1 $\pm$ 7.2	598.0 $\pm$ 11.8	<0.01
Urinary NAG (unit/g creatinine)					
$\geq 8$ (%)	10.6	17.2	21.6	28.8	<0.01
Mean $\pm$ SD	4.5 $\pm$ 3.1	5.6 $\pm$ 4.4	6.1 $\pm$ 5.0	7.9 $\pm$ 6.9	<0.01
Urinary protein (mg/g creatinine)					
$\geq 200$ (%)	14.8	25.5	18.6	37.0	<0.01
Mean $\pm$ SD*	107.5 $\pm$ 2.0	134 $\pm$ 2.1	120.9 $\pm$ 1.9	182.9 $\pm$ 2.4	<0.01
Urinary calcium (mg/g creatinine)					
$\geq 200$ (%)	11.6	12.7	13.4	17.8	0.55
Mean $\pm$ SD*	76.2 $\pm$ 2.4	86.4 $\pm$ 2.3	98.0 $\pm$ 2.0	95.3 $\pm$ 2.3	0.02
Serum creatinine (mg/dl)					
$\geq 1.1$ (%)	36.0	29.0	27.8	39.7	0.10
Mean $\pm$ SD	1.02 $\pm$ 0.30	0.99 $\pm$ 0.26	1.02 $\pm$ 0.42	1.04 $\pm$ 0.33	0.28
Glomerular infiltration rate (ml/min/1.73m <sup>2</sup> )					
< 60 (%)	19.0	19.4	12.4	28.8	0.06
Mean $\pm$ SD	75.4 $\pm$ 19.7	75.2 $\pm$ 20.0	76.9 $\pm$ 21.6	72.0 $\pm$ 20.4	0.46
Hypertension					
Yes (%)	33.4	34.1	28.9	35.6	0.77
Diabetes					
Yes (%)	5.1	6.7	4.1	11.0	0.23
Urinary stone					
Yes (%)	9.6	8.0	7.2	12.3	0.58

\* Geometric mean  $\pm$  standard deviation

**Table 3.** Linear regression analysis of the determinants of each study renal marker

Independent variables	Urinary $\beta_2$ -MG ( $\mu\text{g/g}$ cr)		Urinary NAG (unit/g cr)		Urinary protein (mg/g cr)		Serum creatinine (mg/gdl)		GFR (ml/min/1.73 m <sup>2</sup> )	
	$\beta^*$	p-value	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value	$\beta$	p-value
Age	132.5	<0.01	0.05	<0.01	3.30	<0.01	0.006	<0.01	-0.75	<0.01
Sex	-803.4	0.37	0.29	0.33	25.70	0.07	-0.20	<0.01	-4.92	<0.01
Urinary cadmium	214.5	<0.01	0.16	<0.01	3.44	<0.01	0.00	0.90	0.04	0.64
Hypertension	1,574.8	0.11	0.37	0.26	24.58	0.12	0.08	<0.01	-4.00	<0.01
Diabetes	3,699.6	0.04	3.28	<0.01	112.55	<0.01	0.03	0.47	-2.60	0.28
Urinary stone	7,171.0	<0.01	1.16	0.02	128.06	<0.01	0.21	<0.01	-10.86	<0.01
Adjusted r <sup>2</sup>	0.09		0.16		0.14		0.30		0.37	

\* Regression coefficient

However, urinary cadmium was not significantly associated with serum creatinine or GFR. In the present study, age and the prevalence of urinary stones were

factors significantly associated with those five study renal markers. The prevalence of diabetes was a significant predictor of increased proteinuria

**Table 4.** Logistic regression analysis of the determinants of prevalence of hypertension, diabetes and urinary stones

Dependent variables	Variables in a model*	Odds ratio	95% CI	Coefficient	p-value
Hypertension	Age	1.09	1.07-1.11	0.08	<0.01
	Sex	1.35	0.91-2.02	0.30	0.14
	Smoking	0.92	0.60-1.42	-0.08	0.71
	Body mass index	1.14	1.08-1.20	0.13	<0.01
	Urinary cadmium	1.00	0.97-1.02	-0.001	0.93
	Diabetes	1.79	0.88-3.62	0.58	0.11
	Urinary stone	1.32	0.73-2.38	0.28	0.36
Diabetes	Age	1.02	0.99-1.05	0.02	0.24
	Sex**	2.79	1.25-6.19	1.02	0.01
	Smoking	2.42	1.10-5.31	0.88	0.03
	Body mass index	1.11	1.02-1.21	0.10	0.02
	Urinary cadmium	1.02	0.99-1.06	0.02	0.25
	Hypertension	1.75	0.86-3.54	0.56	0.12
Urinary stone	Age	1.02	1.00-1.04	0.02	0.04
	Sex	2.21	1.28-3.82	0.79	<0.01
	Urinary cadmium	0.99	0.94-1.03	-0.01	0.54
	Urinary calcium	1.00	0.99-1.00	-0.002	0.38

\*Age (years), sex (male/female), smoking (ever/never), body mass index (kg/m<sup>2</sup>), urinary cadmium (µg/g creatinine), diabetes (yes/no), urinary stone (yes/no), hypertension (yes/no), and urinary calcium (mg/g creatinine)

\*\*Female/male

(β<sub>2</sub>-MG, NAG, and protein) whereas hypertension was a significant predictor of increased serum creatinine and decreased GFR.

Multiple logistic regression analysis was used to test the association between urinary cadmium and hypertension, diabetes, and urinary stones, after adjusting for other co-variables (Table 4). In the study population, the rates of hypertension, diabetes, and urinary stones did not significantly increase with increasing urinary cadmium levels.

## Discussion

The tubular proteinuria induced by cadmium is characterized by elevated excretion of low molecular weight proteins such as β<sub>2</sub>-MG and enzymes such as NAG<sup>(1-7)</sup>. The present study revealed positive associations between urinary cadmium and excretion of β<sub>2</sub>-MG, NAG, and total protein by both bivariate and multivariate analyses. Increased excretion of urinary total protein in the present study population indicated that cadmium might produce severe tubular damage and/or glomerular dysfunction through a change in the glomerular permeability to larger proteins. In addition to age, some medical conditions such as diabetes and urinary stones were also found to be related to proteinuria. The authors suggest that

cadmium-exposed persons should be screened for other medical conditions causing nephropathy. Early detection of these disorders and appropriate management may reduce the deterioration of renal damage in the population with excessive exposure to cadmium.

The renal effects of cadmium that result in proteinuria may progress and lead to elevated blood creatinine and end-stage renal disease<sup>(1-8)</sup>. In the present study, although urinary cadmium was significantly associated with proteinuria, it did not show a significant correlation with increased serum creatinine or decreased GFR. These findings indicated that impaired glomerular function might not be severe in the present study population and confirmed that proteinuria should be used as an early sign of nephropathy in persons exposed to cadmium.

An increase in urinary calcium has been reported in some populations exposed to cadmium but not in others<sup>(1-7,16-18)</sup>. Hypercalciuria can result in urinary stone formation in some workers occupationally exposed to cadmium<sup>(16,17)</sup>. Hypercalciuria was not highly prevalent in the presented exposed persons and was not significantly associated with urinary cadmium. Low urinary calcium excretion might partly be due to low calcium intake, which was very common in rural



Thailand<sup>(19)</sup>. Increased excretion of urinary calcium therefore may not be a good sensitive biomarker of renal dysfunction induced by cadmium for the presented Thai population.

An additional renal effect of cadmium observed in workers occupationally exposed to cadmium is an increased frequency of urinary stone formation<sup>(16,17,20,21)</sup>. This effect is likely to be secondary to disruption of calcium metabolism due to kidney damage. In the present study population with environmental cadmium exposure, both urinary cadmium and calcium were not found to significantly correlate with the prevalence of urinary stones. However, the disease was prevalent and was a significant predictor of increased serum creatinine and decreased GFR, which might lead to end-stage renal disease. Therefore, screening for urinary stones should be included in the assessment program for these cadmium-exposed persons. Further studies on comparison between persons living in cadmium-contaminated and non-contaminated areas in the prevalence and analysis of cadmium content of urinary stones may be useful to determine such association.

Some epidemiologic studies have shown a positive association between body cadmium levels and hypertension but some have found no association<sup>(1-3,5,22-24)</sup>. A positive correlation of urinary cadmium with diabetes has been reported recently<sup>(25)</sup>. The present study revealed no significant association between urinary cadmium and hypertension or diabetes by both bivariate and multivariate analyses. Risk for hypertension and diabetes remains uncertain in relation to cadmium exposure and may require further investigations.

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การได้รับสารแคดเมียมในประชากรอำเภอแม่สอด จังหวัดตาก: 3. ความสัมพันธ์ระหว่าง ระดับแคดเมียมในปัสสาวะกับความผิดปกติของไต ความดันโลหิตสูง เบาหวาน และนิ่วในทางเดินปัสสาวะ

วิทยา สวัสดิวุฒิพงศ์, พิสิฐ ลิมปธนโชติ, Muneko Nishio, Ryumon Honda, ปราณิ มหาศักดิ์พันธ์, สมยศ กรินทราทันต์

**วัตถุประสงค์:** เพื่อศึกษาความสัมพันธ์ระหว่างระดับแคดเมียมในปัสสาวะ กับความผิดปกติของไต ความดันโลหิตสูง เบาหวาน และนิ่วในทางเดินปัสสาวะในประชากรผู้ใหญ่ที่อาศัยอยู่ในพื้นที่ ที่มีการปนเปื้อนของสารแคดเมียมในสิ่งแวดล้อม

**วัสดุและวิธีการ:** กลุ่มประชากรตัวอย่างจำนวน 795 ราย ได้รับการตรวจหาระดับแคดเมียมในปัสสาวะ ความผิดปกติของไต ความดันโลหิตสูง เบาหวาน และนิ่วในทางเดินปัสสาวะ สำหรับความผิดปกติของไตนั้น ใช้การตรวจระดับในปัสสาวะของ  $\beta_2$ -microglobulin ( $\beta_2$ -MG), N-acetyl- $\beta$ -D-glucosaminidase (NAG) โปรตีนรวม และแคลเซียมส่วนในเลือด ไซโตครีเอตินิน และคำนวณค่า glomerular infiltration rate (GFR)

**ผลการศึกษา:** ประชากรที่ศึกษามีอายุเฉลี่ยประมาณ 50 ปี พบความชุกของโรคความดันโลหิตสูงร้อยละ 33.3 โรคเบาหวานร้อยละ 6.2 และนิ่วในทางเดินปัสสาวะร้อยละ 8.9 จากการศึกษาพบว่า ร้อยละของประชากรที่ขับโปรตีนในปัสสาวะเพิ่มมากขึ้น พบสูงสุดในกลุ่มที่มีระดับแคดเมียมในปัสสาวะ  $\geq 15$  ไมโครกรัม/กรัมครีเอตินิน และพบว่าเมื่อระดับแคดเมียมในปัสสาวะเพิ่มขึ้น จะมีการขับโปรตีนในปัสสาวะ ( $\beta_2$ -MG, NAG และโปรตีนรวม) เพิ่มสูงขึ้นตามอย่างมีนัยสำคัญทางสถิติ แต่ไม่พบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติ กับระดับแคลเซียมในปัสสาวะ ระดับครีเอตินินในเลือด GFR โรคความดันโลหิตสูง โรคเบาหวาน และนิ่วในทางเดินปัสสาวะ รวมทั้งพบว่าโรคความดันโลหิตสูง โรคเบาหวาน และนิ่วในทางเดินปัสสาวะ เป็นปัจจัยสำคัญที่ทำให้พบความผิดปกติ ของไตมากขึ้น

**สรุป:** การศึกษานี้พบความสัมพันธ์ระหว่างระดับแคดเมียมในปัสสาวะ กับการขับโปรตีนในปัสสาวะ ( $\beta_2$ -MG, NAG และโปรตีนรวม) แต่ไม่พบความสัมพันธ์กับโรคความดันโลหิตสูง โรคเบาหวาน และนิ่วในทางเดินปัสสาวะ

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