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Gender Differences in the Severity of Cadmium Nephropathy

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Abstract: The excretion of β_2-microglobulin (β_2M) above 300 μg/g creatinine, termed tubulopathy, was regarded as the critical effect of chronic exposure to the metal pollutant cadmium (Cd). However, current evidence suggests that Cd may induce nephron atrophy, resulting in a reduction in the estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2. Herein, these pathologies were investigated in relation to Cd exposure, smoking, diabetes, and hypertension. The data were collected from 448 residents of Cd-polluted and non-polluted regions of Thailand. The body burden of Cd, indicated by the mean Cd excretion (E_{Cd}), normalized to creatinine clearance (C_{cr}) as (E_{Cd}/C_{cr}) × 100 in women and men did not differ (3.21 vs. 3.12 μg/L filtrate). After adjustment of the confounding factors, the prevalence odds ratio (POR) for tubulopathy and a reduced eGFR were increased by 1.9-fold and 3.2-fold for every 10-fold rise in the Cd body burden. In women only, a dose–effect relationship was seen between β_2M excretion ($E_{\beta 2M}/C_{cr}$) and E_{Cd}/C_{cr} (F = 3.431, η^2 0.021). In men, $E_{\beta 2M}/C_{cr}$ was associated with diabetes (β = 0.279). In both genders, the eGFR was inversely associated with $E_{\beta 2M}/C_{cr}$. The respective covariate-adjusted mean eGFR values were 16.5 and 12.3 mL/min/1.73 m^2 lower in women and men who had severe tubulopathy (($E_{\beta 2M}/C_{cr}$) × 100 ≥ 1000 μg/L filtrate). These findings indicate that women were particularly susceptible to the nephrotoxicity of Cd, and that the increment of $E_{\beta 2M}/C_{cr}$ could be attributable mostly to Cd-induced impairment in the tubular reabsorption of the protein together with Cd-induced nephron loss, which is evident from an inverse relationship between $E_{\beta 2M}/C_{cr}$ and the eGFR.

Keywords: β_2-microglobulin; cadmium; diabetes; GFR; hypertension; smoking; tubular proteinuria

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1. Introduction

Cadmium (Cd) is a toxic metal pollutant that preferentially accumulates in the proximal tubule of kidneys, where it causes tubular cell injury, cell death, nephron atrophy, and eventually, a reduction in the estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 [1–4]. As exposure to Cd in the diet is inevitable for most populations, it has become an environmental toxicant of significant worldwide public health concern. A total diet study undertaken in Japan between 2013 and 2018 reported that rice and its products, green vegetables, and cereals and seeds plus potatoes constituted 38%, 17%, and 11% of total dietary exposure, respectively [5].

To safeguard against excessive exposure to Cd in the human diet, guidelines, referred to as a tolerable intake level of Cd, were created by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) [6]. Notably, the "tolerable" intake level was based on the risk assessment model that assumed tubular proteinuria, reflected by

an increase in the excretion of the low-molecular-weight protein β2-microglubulin (β_2M, $E_{\beta 2M}$) above 300 μg/g creatinine, to be an early warning sign of the nephrotoxicity of Cd. Consequently, tubulopathy is the most frequently reported adverse effect of Cd exposure. Numerous studies, however, have cast considerable doubt on the utility of $E_{\beta 2M}$ for such purposes.

This study aims to examine whether the exposure to Cd adversely impacts kidney toxicity differently in men and women. To this end, tubular dysfunction and changes in the eGFR were quantified in residents of Cd-polluted and non-polluted regions of Thailand and analyzed in relation to Cd exposure levels. The confounding impact of smoking, diabetes, and hypertension were also evaluated. The exposure to Cd was assessed via the measurement of blood Cd concentration ($[Cd]_b$) and urinary Cd excretion (E_{Cd}). Tubular dysfunction was assessed via $E_{\beta 2M}$. The equations developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) were used to compute the estimated GFR (eGFR) [7].

2. Materials and Methods

2.1. Participants

To obtain a group with a wide range of environmental Cd exposure amenable to dose–effect relationship assessment, we assembled data from 334 women and 114 men who participated in the cross-sectional studies conducted in a high-exposure area of the Mae Sot district, Tak province [8], and a low-exposure locality in Pakpoon Municipality of Nakhon Si Thammarat Province [9]. Based on the data from a nationwide survey of Cd levels in soils and food crops [10], environmental exposure to Cd in Nakhon Si Thammarat was low.

The study protocol for the Mae Sot group was approved by the Institutional Ethical Committees of Chiang Mai University and the Mae Sot Hospital [8]. The study protocol for the Nakhon Si Thammarat group was approved by the Office of the Human Research Ethics Committee of Walailak University in Thailand [9].

All participants gave informed consent prior to participation. They had been living at their current addresses for at least 30 years. Exclusion criteria were pregnancy, breast-feeding, a history of metal work, and a hospital record or physician's diagnosis of an advanced chronic disease. Diabetes was defined as having fasting plasma glucose levels $\geq$ 126 mg/dL (https://www.cdc.gov/diabetes/basics/getting-tested.html (accessed on 25 June 2023)) or a physician's prescription of anti-diabetic medications. Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg, diastolic blood pressure $\geq$ 90 mmHg [11], a physician's diagnosis, or prescription of anti-hypertensive medications.

2.2. Collection and Analysis of Blood and Urine Samples

Second morning urine samples were collected after an overnight fast, and whole blood samples were obtained within 3 h after the urine sampling. Aliquots of urine, whole blood, and plasma were stored at −20 or −80 °C prior to analysis. The assay for urine and plasma concentrations of creatinine ($[cr]_u$ and $[cr]_p$) was based on the Jaffe reaction. The assay of urinary β_2M concentration ($[\beta_2M]_u$) was based on the latex immunoagglutination method (LX test, Eiken 2MGII; Eiken and Shionogi Co., Tokyo, Japan) or the ELISA method (Sino Biological Inc., Wayne, PA, USA).

Urinary Cd concentrations ($[Cd]_u$) were determined using an atomic absorption spectrophotometer. Urine standard reference material No. 2670 (National Institute of Standards, Washington, DC, USA) or the reference urine metal control levels 1, 2, and 3 (Lyphocheck, Bio-Rad, Hercules, CA, USA) were used for quality control, analytical accuracy, and precision assurance. The limit of detection (LOD) of Cd quantitation was defined as 3 times the standard deviation of blank measurements. When $[Cd]_u$ was below its detection limit (0.1 μg/L), the Cd concentration assigned was the LOD divided by the square root of 2 [12].

2.3. Estimated Glomerular Filtration Rates (eGFRs)

The GFR is the product of the nephron number and mean single nephron GFR, and in theory, the GFR is indicative of nephron function [13–15]. In practice, the GFR is estimated from established chronic kidney disease epidemiology collaboration (CKD-EPI) equations and is reported as the eGFR [15].

Male eGFR = 141 × [plasma creatinine/0.9]^{Y} × 0.993^{age}, where Y = −0.411 if $[cr]_p \leq 0.9$ mg/dL, and Y = −1.209 if $[cr]_p > 0.9$ mg/dL. Female eGFR = 144 × [plasma creatinine/0.7]^{Y} × 0.993^{age}, where Y = −0.329 if $[cr]_p \leq 0.7$ mg/dL, and Y = −1.209 if $[cr]_p > 0.7$ mg/dL. CKD stages 1, 2, 3a, 3b, 4, and 5 corresponded to eGFRs of 90–119, 60–89, 45–59, 30−44, 15–29, and <15 mL/min/1.73 m^2, respectively.

2.4. Normalization of Excretion Rate

E_x was normalized to E_{cr} as $[x]_u/[cr]_u$, where x = Cd or β_2M; $[x]_u$ = urine concentration of x (mass/volume); and $[cr]_u$ = urine creatinine concentration (mg/dL). The ratio $[x]_u/[cr]_u$ was expressed in µg/g of creatinine.

E_x was normalized to C_{cr} as $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd or β_2M; $[x]_u$ = urine concentration of x (mass/volume); $[cr]_p$ = plasma creatinine concentration (mg/dL); and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/C_{cr} was expressed as the excretion of x per volume of filtrate [7].

2.5. Statistical Analysis

Data were analyzed using IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). The Mann–Whitney U test was used to assess differences in mean values in women and men, and Pearson's chi-squared test was used to assess differences in percentages. The one-sample Kolmogorov–Smirnov test was used to identify departures of continuous variables from a normal distribution, and logarithmic transformation was applied to variables that showed rightward skewing before they were subjected to parametric statistical analysis.

The multivariable logistic regression analysis was used to determine the prevalence odds ratio (POR) for categorical outcomes. Reduced eGFR was assigned when eGFR ≤ 60 mL/min/1.73 m^2. For C_{cr}-normalized data, tubular dysfunction was defined as $(E_{\beta 2M}/C_{cr}) \times 100 \geq 300$ µg/L of filtrate. For E_{cr}-normalized data, tubular dysfunction was defined as $E_{\beta 2M}/E_{cr} \geq 300$ µg/g creatinine [6]. Univariate analysis of covariance via Bonferroni correction in multiple comparisons was used to obtain covariate-adjusted mean E_{Cd}/C_{cr} and mean $E_{\beta 2M}/C_{cr}$. For all tests, p-values ≤ 0.05 were considered to indicate statistical significance.

3. Results

3.1. Descriptive Characteristics of Participants

This cohort consisted of 334 women (mean age 51.5 years) and 114 men (mean age 49.9 years) (Table 1).

Table 1. Characteristics of study subjects.

Parameters	All Subjects, *n* = 448	Women, *n* = 334	Men, *n* = 114	*p*
Age, years	51.1 ± 8.6	51.5 ± 9.0	49.9 ± 7.2	0.344
BMI, kg/m^2	24.8 ± 4.0	25.2 ± 4.0	23.7 ± 3.6	<0.001
Smoking, %	31.3	18.6	68.4	<0.001
Hypertension, %	48.7	50.6	43.0	0.160
Diabetes, %	15.4	16.2	13.2	0.442
eGFR [a], mL/min/1.73 m^2	90 ± 18	89 ± 19	93 ± 16	0.145
Reduced eGFR [b], %	6.9	8.1	3.5	0.097
Plasma creatinine, mg/dL	0.82 ± 0.22	0.78 ± 0.21	0.95 ± 0.21	<0.001
Urine creatinine, mg/dL	114 ± 74	108 ± 74	132 ± 72	<0.001

Table 1. *Cont.*

Parameters	All Subjects, *n* = 448	Women, *n* = 334	Men, *n* = 114	*p*
Blood Cd, μg/L	2.75 ± 3.19	2.58 ± 3.10	3.25 ± 3.41	0.038
Urine Cd, μg/L	4.22 ± 5.67	4.36 ± 6.14	3.82 ± 4.01	0.875
Urine β_2M, μg/L	3122 ± 18,836	2596 ± 17,238	4665 ± 22,903	0.544
Normalized to C_{cr} (E_x/C_{cr}) [c]				
(E_{Cd}/C_{cr}) × 100, μg/L filtrate	3.19 ± 3.72	3.21 ± 3.79	3.12 ± 3.55	0.639
($E_{\beta 2M}/C_{cr}$) × 100, μg/L filtrate	3839 ± 30,422	3078 ± 26,986	6072 ± 38,837	0.212
($E_{\beta 2M}/C_{cr}$) × 100, μg/L filtrate, %				
<300	41.1	38.9	47.4	
300−999	34.8	35.9	31.6	
≥1000	24.1	25.1 *	21.1 ***	
Normalized to E_{cr} (E_x/E_{cr}) [d]				
E_{Cd}/E_{cr}, μg/g creatinine	4.02 ± 4.41	4.26 ± 4.62	3.30 ± 3.68	0.028
$E_{\beta 2M}/E_{cr}$, μg/g creatinine	3220 ± 21,847	3005 ± 22,812	3850 ± 18,815	0.017
$E_{\beta 2M}/E_{cr}$, μg/g creatinine, %				
<300	35.5	32.3	45.6	
300−999	34.6	34.7	34.2	
≥1000	29.7	32.9	20.2 **	

n, number of subjects; BMI, body mass index; eGFR, estimated glomerular filtration rate; β_2M, β_2-microglobulin; E_x, excretion of x; cr, creatinine; C_{cr}, creatinine clearance; Cd, cadmium; [a] eGFR was determined by established CKD-EPI equations [15]; [b] reduced eGFR corresponds to eGFR ≤ 60 mL/min/1.73 m^2; [c] $E_x/E_{cr} = [x]_u/[cr]_u$; [d] $E_x/C_{cr} = [x]_u[cr]_p/[cr]_u$, where x = Cd or β_2M [7]. Data for all continuous variables are arithmetic means ± standard deviation (SD). For all tests, $p \leq 0.05$ identifies statistical significance, determined by Pearson's chi-squared test for % differences and by the Mann–Whitney U test for mean differences between women and men. * $p = 0.005$; ** $p = 0.004$; *** $p = 0.002$.

Of the total of 334 women, 224 and 110 were from the high- and low-exposure regions, respectively. In comparison, of the total 114 men, 84 and 30 males were from the high- and low-exposure regions, respectively.

The respective overall percentages of smoking, hypertension, diabetes, and reduced eGFR were 31.3%, 48.7%, 15.4%, and 6.9%. More than half of the men (68.4%) smoked cigarettes, while only 18.6% of women did. The % of all other ill health conditions in men and women did not differ, nor did their mean age differ.

With the exception of BMI, the mean plasma creatinine, mean urine creatinine, and mean blood Cd were all lower in women than in men. The mean eGFR, mean urine Cd, and mean β_2M concentrations in women and men were not statistically different.

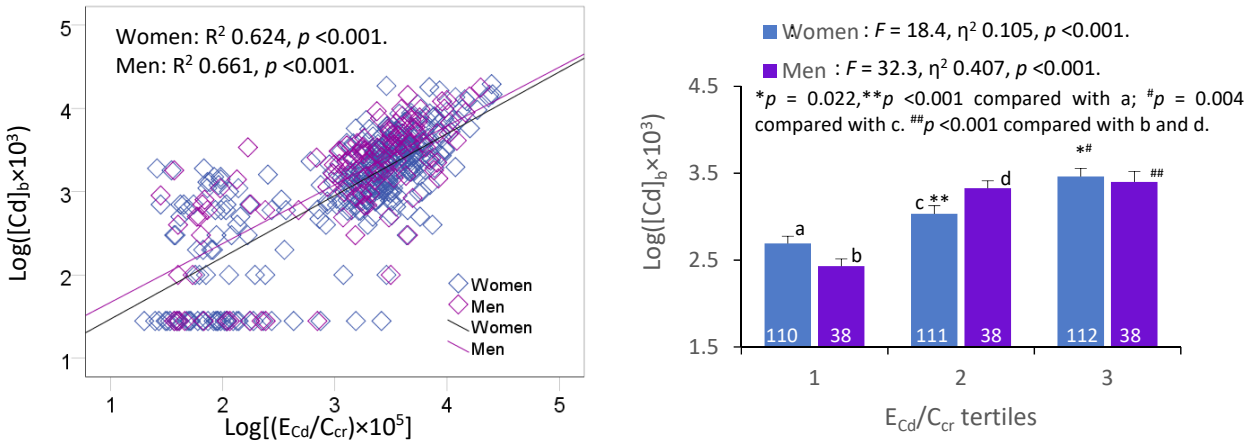
For the C_{cr}-normalized data, the mean E_{Cd}/C_{cr} and mean $E_{\beta 2M}/C_{cr}$ in women and men both did not differ statistically. However, there were statistically significant differences in the % of women and men across three $E_{\beta 2M}/C_{cr}$ groups.

For the E_{cr}-normalized data, the mean E_{Cd}/E_{cr} and mean $E_{\beta 2M}/E_{cr}$ were higher in women than in men. The % of men across three $E_{\beta 2M}/E_{cr}$ groups differed, but there was no difference in the % of women across the $E_{\beta 2M}/E_{cr}$ groups.

3.2. Cadmium Exposure Characterization

Figure 1 provides scatterplots relating two Cd exposure indicators, namely the blood Cd concentration and the excretion rate of Cd, represented as E_{Cd}/C_{cr}.

A strong positive association between $\log([Cd]_b \times 10^3)$ and $[\log[(E_{Cd}/C_{cr}) \times 10^5]$ was evident in both women and men (Figure 1a). After the adjustment for the covariates and interactions, the Cd body burden ($[\log[(E_{Cd}/C_{cr}) \times 10^5]$ explained a larger proportion of the variation in the blood Cd concentrations ($\log([Cd]_b \times 10^3)$ in men ($\eta^2 = 0.407$) than in women ($\eta^2 = 0.105$) (Figure 1b).



(**a**) (**b**)

Figure 1. Blood cadmium and urinary cadmium excretion relationship. Scatterplots relating log($[Cd]_b \times 10^3$] to log[(E_{Cd}/C_{cr}) × 10^5] in women and men (**a**). Coefficients of determination (R^2) and *p*-values are provided for all scatterplots. Bar graph (**b**) depicts mean log($[Cd]_b \times 10^3$] values in women and men across E_{Cd}/C_{cr} tertiles. Letters a and c refer to groups of women whose $E_{Cd}/C_{c}r$ values were in low and middle E_{Cd}/C_{cr} tertiles, respectively. Letters b and d refer to groups of men whose E_{Cd}/C_{cr} values were in low and middle E_{Cd}/C_{cr} tertiles, respectively. All means were obtained via univariate analysis with adjustment for covariates and interactions. For women, respective arithmetic means and standard deviations (SD) for (E_{Cd}/C_{cr}) × 100 tertiles 1, 2, and 3 are 0.37 (0.47), 2.34 (0.52), and 6.84 (4.46) µg/L of filtrate. For men, respective arithmetic means and standard deviations (SD) for (E_{Cd}/C_{cr}) × 100 tertiles quartiles 1, 2, and 3 are 0.36 (0.42), 2.14 (0.63), and 6.81 (3.78) µg/L of filtrate. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

To further address the variables/factors that may influence the blood Cd levels, we conducted multiple regression and univariate analyses of variance that incorporated age, BMI, log[(E_{Cd}/C_{cr}) × 10^5], smoking, diabetes, and hypertension as independent variables. Table 2 provides the results of these analyses.

Table 2. Determinants of blood cadmium concentration in women versus men.

Independent Variables/Factors	Log($[Cd]_b \times 10^3$), µg/L					
	Women, *n* = 334			Men, *n* = 114		
	β	η^2	*p*	β	η^2	*p*
Age, years	−0.170	0.057	<0.001	−0.004	1×10^{-6}	0.946
BMI, kg/m²	−0.019	0.001	0.586	−0.079	0.022	0.180
Log[(E_{Cd}/C_{cr}) × 10^5], µg/L filtrate	0.619	0.367	<0.001	0.581	0.420	<0.001
Smoking	0.123	0.028	0.001	0.184	0.055	0.002
Diabetes	−0.053	0.010	0.182	−0.246	0.095	<0.001
Hypertension	0.048	0.007	0.162	−0.061	0.004	0.287
DM × HTN	n/a	0.016	0.023	n/a	n/a	n/a
SMK × DM × HTN	n/a	n/a	n/a	n/a	0.042	0.036
Adjusted R^2	0.624	n/a	<0.001	0.661	n/a	<0.001

β, standardized regression coefficient; adjusted R^2, coefficient of determination; DM, diabetes; HTN, hypertension; SMK, smoking; n/a, not applicable. β indicates strength of association of log($[Cd]_b \times 10^3$) with independent variables (first column). Adjusted R^2 indicates a fractional variation of log($[Cd]_b \times 10^3$) explained by all independent variables. Eta square (η^2) indicates the fraction of the variability of each dependent variable explained by a corresponding independent variable. *p*-values ≤ 0.05 indicate a statistically significant contribution of variation of an independent variable to variation of a dependent variable.

In women, higher $[Cd]_b$ values were strongly associated with higher E_{Cd}/C_{cr} (β = 0.619), and were moderately associated with smoking (β = 0.123) and younger age (β = −0.170). E_{Cd}/C_{cr}, age, and smoking explained, respectively, 36.7%, 5.7%, and 2.8% of the variation of $[Cd]_b$ in women, while the interaction between diabetes and hypertension contributed

to 1.6% of the $[Cd]_b$ variability. In men, higher $[Cd]_b$ values were strongly associated with higher E_{Cd}/C_{cr} (β = 0.581), and were moderately associated with smoking (β = 0.184) and not having diabetes (β = −0.246). E_{Cd}/C_{cr}, smoking, and diabetes accounted, respectively, for 42%%, 5.5%, and 9.5% of the variation of $[Cd]_b$ in men, while the interaction between smoking, diabetes, and hypertension contributed to 4.2% of the $[Cd]_b$ variability.

3.3. Effects of Cadmium Exposure on β_2M Excretion

We assessed the effects of Cd exposure on Eβ_2M using multiple linear regression and univariate/covariance analyses, where the indicators of Cd exposure ($[Cd]_b$ and E_{Cd}) were incorporated as the independent variables together with age, BMI, smoking, diabetes, and hypertension (Table 3).

Table 3. Associations of β_2-mcirogloubulin excretion with cadmium exposure measurements.

Independent Variables/Factors	Log[($E_{\beta 2M}/C_{cr}$) × 10^3], µg/L Filtrate					
	All Subjects		Women		Men	
	β	*p*	β	*p*	β	*p*
Age, years	0.137	0.013	0.131	0.041	0.128	0.238
BMI, kg/m^2	−0.089	0.065	−0.102	0.062	−0.043	0.664
Log($[Cd]_b \times 10^3$), µg/L filtrate	−0.016	0.824	−0.083	0.328	0.217	0.180
Log[(E_{Cd}/C_{cr}) × 10^5], µg/L filtrate	0.283	<0.001	0.306	<0.001	0.175	0.247
Gender	0.052	0.318	–	–	–	–
Smoking	0.063	0.255	0.093	0.094	−0.065	0.526
Diabetes	0.323	<0.001	0.349	<0.001	0.279	0.017
Hypertension	0.015	0.745	−0.023	0.660	0.142	0.139
Adjusted R^2	0.105	<0.001	0.125	<0.001	0.059	0.060

β, standardized regression coefficient; adjusted R^2, coefficient of determination. β indicates strength of association of log[($E_{\beta 2M}/C_{cr}$) × 10^3] with independent variables (first column). Adjusted R^2 indicates a fractional variation of log[($E_{\beta 2M}/C_{cr}$) × 10^3] explained by all independent variables. For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of an independent variable to log[($E_{\beta 2M}/C_{cr}$) × 10^3] variability.

In all subjects, $E_{\beta 2M}/C_{cr}$ was associated with age (β = 0.137), E_{Cd}/C_{cr} (β = 0.283), and diabetes (β = 0.323). In women, the associations of $E_{\beta 2M}/C_{cr}$ with these three independent variables were evident. In men, $E_{\beta 2M}/C_{cr}$ only showed a significant association with diabetes (β = 0.279).

We next examined the association between the E_{Cd}/C_{cr} and $E_{\beta 2M}/C_{cr}$ with the scatterplots and the covariate-adjusted mean $E_{\beta 2M}$ in subjects grouped by E_{Cd}/C_{cr} tertiles (Figure 2).

The relationship between $E_{\beta 2M}/C_{cr}$ and E_{Cd}/C_{cr} was weak and statistically insignificant in all subjects (Figure 1a), as well as in women and men (Figure 2c). However, with the adjustment for the covariates that included age and BMI, diabetes, hypertension, and smoking (Figure 2b), a significant contribution of the Cd body burden to the variability of $E_{\beta 2M}$ became evident when all subjects were included in an analysis (F = 4.473, η^2 0.012, p = 0.021). The $E_{\beta 2M}$ in the subjects of the high E_{Cd}/C_{cr} tertile was higher compared with those of the middle and low E_{Cd}/C_{cr} tertiles (Figure 1b). In the subgroup analysis (Figure 1d), a dose–effect relationship of E_{Cd} and $E_{\beta 2M}$ was seen in women only (F = 3.431, η^2 0.021, p = 0.034).

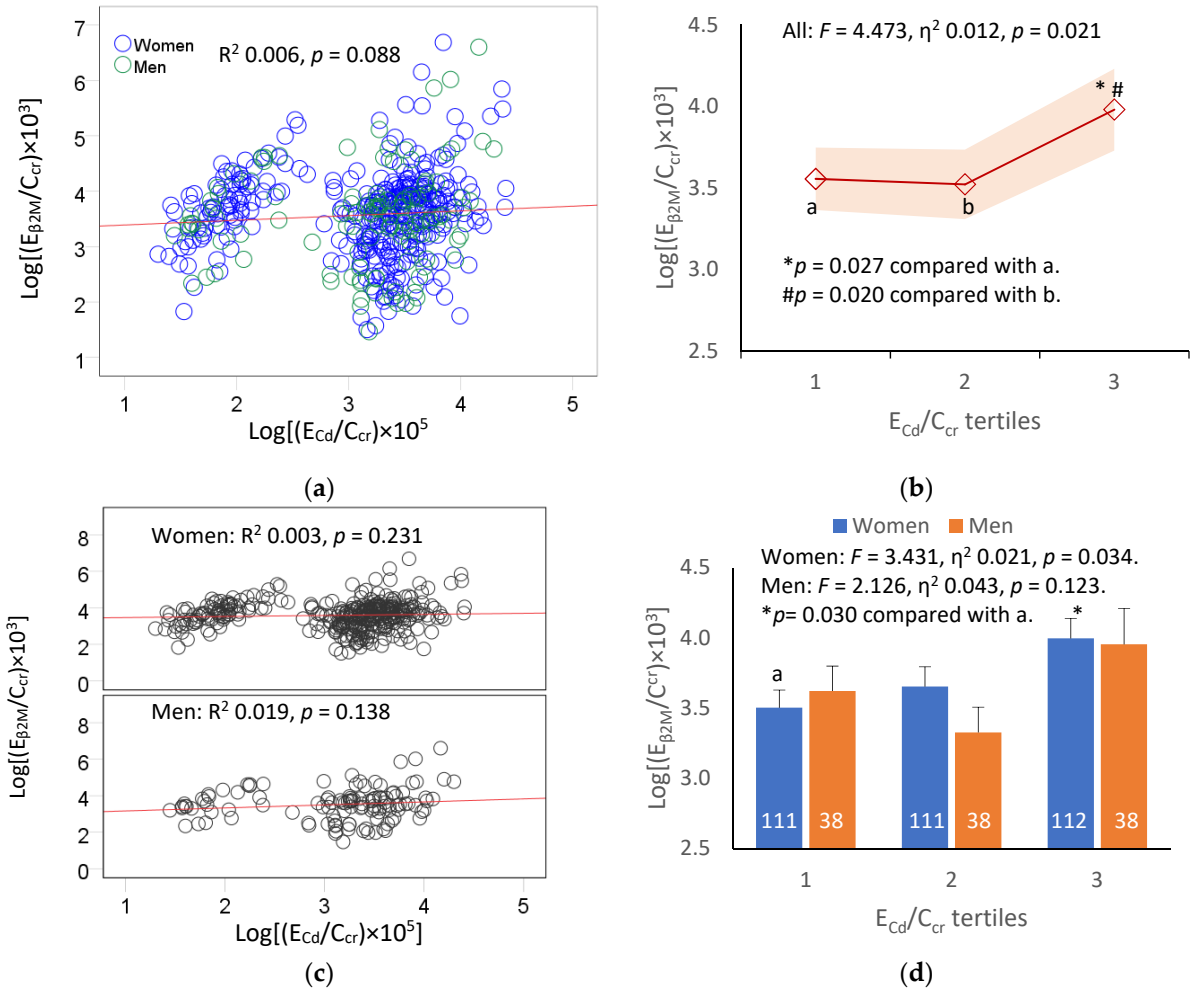


Figure 2. Dose–effect relationship of β_2-microgloubulin and cadmium excretion. Scatterplots relate $\log[(E_{\beta M}/C_{cr}) \times 10^3]$ to $\log[(E_{Cd}/C_{cr}) \times 10^5]$ in all subjects (**a**), and in women and men (**c**). Coefficients of determination (R^2) and *p*-values are provided for all scatterplots. The color-coded area graph (**b**) depicts means of $\log[(E_{\beta 2M}/C_{cr}) \times 10^3]$ across E_{Cd}/C_{cr} tertiles. Shaded areas indicate variability of means. Bar graph (**d**) depicts mean $\log[(E_{\beta 2M}/C_{cr}) \times 10^3]$ in women and men in each E_{Cd}/C_{cr} tertile. The respective numbers of subjects in E_{Cd}/C_{cr} tertiles 1, 2, and 3 are 149, 149, and 150. In Figure 2d, a letter a refers to a group of women whose ECd/C_{cr} values were in low E_{Cd}/C_{cr} tertile. All means were obtained via univariate analysis with adjustment for covariates and interaction. For women, respective arithmetic means and standard deviations (SD) for $(E_{Cd}/C_{cr}) \times 100$ tertiles 1, 2, and 3 are 0.37 (0.47), 2.34 (0.52), and 6.84 (4.46) μg/L of filtrate. For men, respective arithmetic means and standard deviations (SD) for $(E_{Cd}/C_{cr}) \times 100$ tertiles 1, 2, and 3 are 0.36 (0.42), 2.14 (0.63), and 6.81 (3.78) μg/L of filtrate. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

3.4. Effects of Cadmium Exposure on the Prevalence Odds of Tubulopathy

Table 4 provides the results of the logistic regression analysis of abnormal $E_{\beta 2M}$ that incorporated age, BMI, $\log[(E_{Cd}/C_{cr}) \times 10^5]$, gender, smoking, diabetes, and hypertension as independent variables.

Among seven independent variables, the prevalence odds ratios (POR) for $(E_{\beta 2M}/C_{cr}) \times 100 \geq 300$–999 and ≥1000 μg/L filtrate were increased with age, $\log[(E_{Cd}/C_{cr}) \times 10^5]$, and diabetes. All of the other four independent variables did not show a significant association with abnormal β_2M excretion. For every 10-fold rise in E_{Cd}/C_{cr}, the POR for $(E_{\beta 2M}/C_{cr}) \times 100$ of ≥300 and ≥1000 μg/L were increased by 1.94-fold and 3.34-fold, respectively.

Table 4. Prevalence odds for excessive excretion of β_2M in relation to cadmium excretion and other variables.

Independent Variables/Factors	Number of Subjects	$(E_{\beta 2M}/C_{cr}) \times 100 \geq 300$ μg/L		$(E_{\beta 2M}/C_{cr}) \times 100 \geq 1000$ μg/L	
		POR (95% CI)	*p*	POR (95% CI)	*p*
Age, years	448	1.036 (1.007, 1.067)	0.016	1.062 (1.027, 1.098)	<0.001
BMI, kg/m^2	448	0.971 (0.919, 1.025)	0.284	0.958 (0.896, 1.023)	0.203
Log[$(E_{Cd}/C_{cr}) \times 10^5$], μg/L filtrate	448	1.940 (1.344, 2.802)	<0.001	3.343 (2.036, 5.488)	<0.001
Gender (F/M)	334/114	1.406 (0.835, 2.367)	0.200	1.299 (0.687, 2.458)	0.421
Smoking	140	1.067 (0.645, 1.765)	0.801	1.388 (0.763, 2.522)	0.282
Diabetes	69	5.294 (2.526, 11.09)	<0.001	11.52 (5.004, 26.50)	<0.001
Hypertension	218	1.066 (0.714, 1.592)	0.753	1.535 (0.942, 2.501)	0.085

POR, prevalence odds ratio; CI, confidence interval. The units of $(E_{\beta 2M}/C_{cr}) \times 100$ and log[$(E_{Cd}/C_{cr}) \times 10^5$] are μg/L filtrate; data were generated from logistic regression analyses, relating POR for excessive β_2M excretion to seven independent variables (first column). For all tests, *p*-values ≤ 0.05 indicate a statistically significant association of POR with a given independent variable.

3.5. Effects of Cadmium Exposure on eGFR

Similarly, we assessed the effects of Cd exposure on the estimated glomerular filtration rate (eGFR) via multiple linear regression and logistic regression analyses, where $[Cd]_b$ and E_{Cd} were incorporated as the independent variables together with age, BMI, smoking, diabetes, and hypertension (Table 5).

Table 5. Associations of eGFR with cadmium exposure measurements and other variables.

Independent Variables/Factors	eGFR, mL/min/1.73 m^2					
	All Subjects		Women		Men	
	β	*p*	β	*p*	β	*p*
Age, years	−0.517	<0.001	−0.511	<0.001	−0.506	<0.001
BMI, kg/m^2	−0.064	0.136	−0.048	0.327	−0.149	0.095
Log($[Cd]_b \times 10^3$), μg/L filtrate	0.053	0.420	0.102	0.182	−0.153	0.291
Log[$(E_{Cd}/C_{cr}) \times 10^5$], μg/L filtrate	−0.148	0.026	−0.185	0.018	0.011	0.933
Gender	−0.001	0.977	–	–	–	–
Smoking	0.025	0.610	0.018	0.717	0.071	0.438
Diabetes	−0.109	0.023	−0.128	0.021	−0.055	0.593
Hypertension	−0.079	0.055	−0.050	0.295	−0.212	0.014
Adjusted R^2	0.279	<0.001	0.281	<0.001	0.249	<0.001

eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; adjusted R^2, coefficient of determination. β indicates strength of association of eGFR with independent variables (first column). Adjusted R^2 indicates a fractional variation of eGFR explained by all independent variables. For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of an independent variable to eGFR variability.

In all subjects, the eGFR was inversely associated with age (β = −0.517), E_{Cd} (β = −0.148), and diabetes (β = −0.109). In the subgroup analysis, inverse associations of the eGFR with these three independent variables (age, E_{Cd}, and diabetes) were seen only in women. In men, the eGFR was not associated with E_{Cd}, but this parameter showed inverse associations with age (β = −0.506) and hypertension (β = −0.212).

In the logistic regression of a reduced eGFR (eGFR $\leq$ 60 mL/min/1.73 m^2), age, BMI, log[$(E_{Cd}/C_{cr}) \times 10^5$], gender, smoking, diabetes, and hypertension were incorporated as independent variables (Table 6).

The POR values for a reduced eGFR were increased with age, log[$(E_{Cd}/C_{cr}) \times 10^5$], and diabetes. For every 10-fold rise in E_{Cd}/C_{cr}, the POR for a reduced eGFR was increased by 3.2-fold. There was a 4.2-fold increase in the POR for a reduced eGFR among those with diabetes.

Table 6. Prevalence odds for a reduced eGFR in relation to cadmium excretion and other variables.

Independent Variables/ Factors	Reduced eGFR [a]				
	β Coefficients (SE)	POR	95% CI		*p*
			Lower	Upper	
Age, years	0.136 (0.027)	1.146	1.086	1.209	<0.001
BMI, kg/m^2	0.020 (0.051)	1.021	0.923	1.128	0.688
Log[(E_{Cd}/C_{cr}) × 10^5], µg/L filtrate	1.154 (0.358)	3.172	1.572	6.402	0.001
Gender	−0.542 (0.649)	0.582	0.163	2.075	0.404
Smoking	−0.228 (0.583)	0.796	0.254	2.493	0.695
Diabetes	1.439 (0.524)	4.217	1.510	11.78	0.006
Hypertension	0.115 (0.427)	1.122	0.486	2.591	0.787

[a] Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73 m^2; β, regression coefficient; POR, prevalence odds ratio; SE, standard error of mean; CI, confidence interval. Data were generated from logistic regression, relating POR for a reduced eGFR to seven independent variables (first column). For each test, *p*-values ≤ 0.05 indicate a statistically significant contribution of individual independent variables to the POR for a reduced eGFR.

3.6. Inverse Relationship of β_2M Excretion and eGFR

Figure 3 provides scatterplots relating the eGFR to $E_{\beta 2M}$ among the study subjects together with the covariate-adjusted means of the eGFR in women and men.

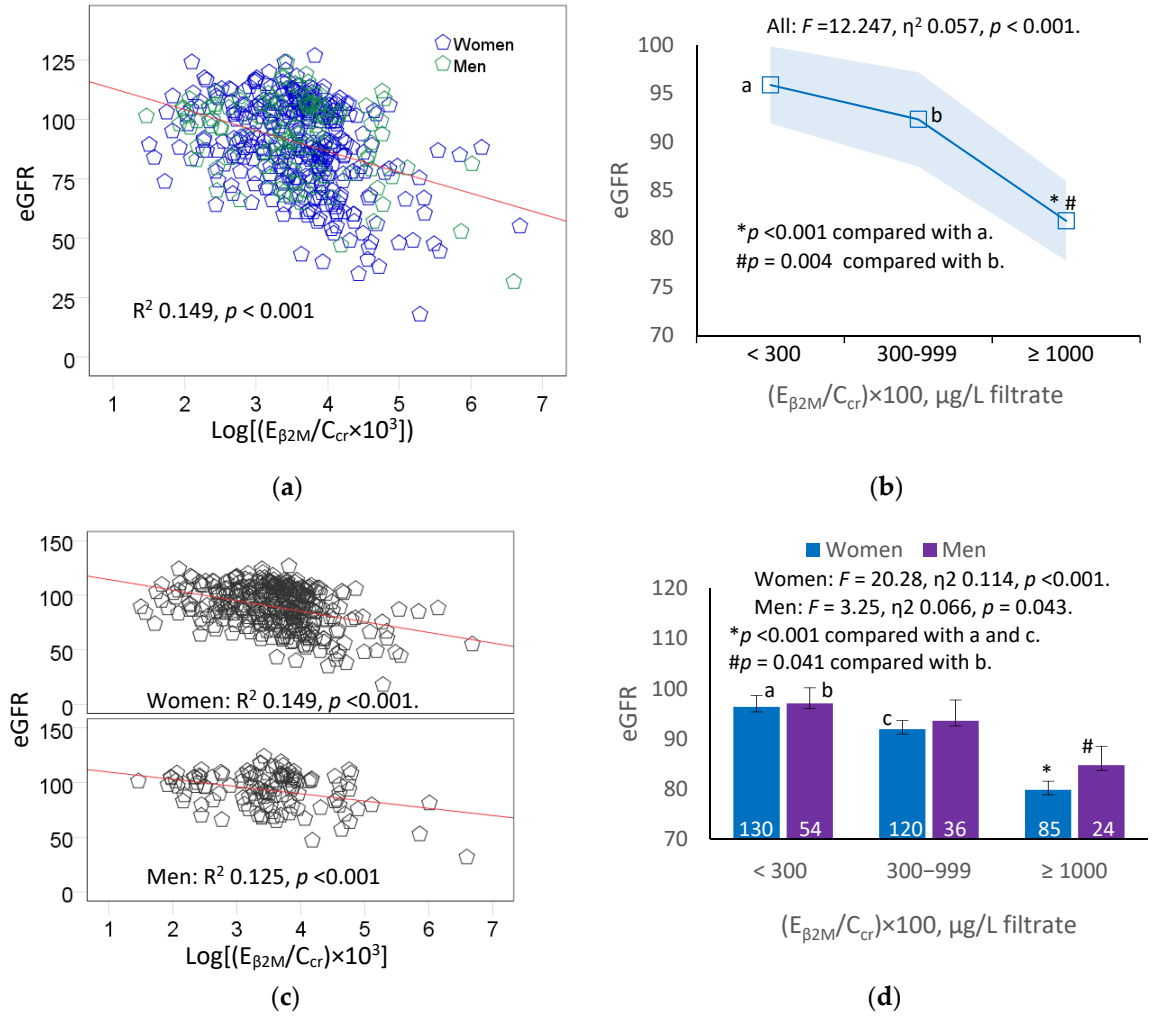


Figure 3. An inverse relationship of eGFR with β_2-microgloubulin excretion. Scatterplots relate eGFR to log[($E_{\beta M}/C_{cr}$) × 10^3] in all subjects (**a**), and in women and men (**c**). Coefficients of determination

(R^2) and *p*-values are provided for all scatterplots. The color-coded area graph (**b**) depicts means of eGFR across three $E_{\beta 2M}/C_{cr}$ ranges. Shaded areas indicate variability of means. Bar graph (**d**) depicts means of eGFR in women and men in each $E_{\beta 2M}/C_{cr}$ range. The respective numbers of subjects in $(E_{\beta 2M}/C_{cr}) \times 100 < 300$, 300−999 and ≥1000 µg/L of filtrate are 184, 156, and 109. In Figure 3d, letters a and c refer to groups of women whose $(E_{\beta 2M}/C_{cr}) \times 100 < 300$ and 300–999 µg/L filtrate, respectively. The letter b refers to a group of men whose $(E_{\beta 2M}/C_{cr}) \times 100 < 300$ µg/L filtrate, respectively. All means were obtained via univariate analysis with adjustment for covariates and interaction. For all tests, *p*-values ≤ 0.05 indicate statistically significant differences.

A statistically significant inverse relationship between the eGFR and $E_{\beta 2M}$ was seen in all subjects (Figure 3a), as well as in women and men (Figure 3c). In all subjects (Figure 3b), the eGFR explained 5.7% of the variation in $E_{\beta 2M}/C_{cr}$ ($F = 12.247$, $p < 0.001$).

For simplicity, the degree of tubulopathy, assessed via $E_{\beta 2M,}$ was graded into three levels, where levels 1, 2, and 3 of tubulopathy corresponded to $(E_{\beta 2M}/C_{cr}) \times 100 < 300$, 300–999, and ≥1000 µg/L filtrate, respectively.

The covariate-adjusted mean eGFR was 14.0 and 10.5 mL/min/1.73 m^2 lower in subjects with level 3 tubulopathy, compared to those with tubular dysfunction levels 2 and 1, respectively (Figure 3b).

In the subgroup analysis (Figure 3d), the η^2 values indicated a nearly twice larger effect size of the eGFR on $E_{\beta 2M}$ in women ($\eta^2 = 0.114$), compared to men ($\eta^2 = 0.066$). In women, those with level 3 tubulopathy had a covariate-adjusted mean eGFR 16.5 and 12.0 mL/min/1.73 m^2 lower compared to those with levels 1 and 2 tubulopathy, respectively. In men, those with level 3 tubulopathy had a covariate-adjusted mean eGFR 12.3 mL/min/1.73 m^2 lower compared to those with level 1 tubulopathy. The adjusted mean eGFR values in men with tubulopathy levels 3 and 2 did not differ statistically.

In another logistic regression, a relative contribution of Cd exposure and tubular dysfunction levels to the prevalence of a reduced eGFR was determined. E_{Cd} was entered as a continuous variable, while $E_{\beta 2M}$ was categorized into levels 1, 2, and 3, as previously stated. Table 7 provides the results of such analysis.

The POR values for a reduced eGFR rose with age (POR = 1.14), E_{Cd}/C_{cr} (POR = 2.25), tubulopathy level 2 (POR = 8.31), and tubulopathy level 3 (POR = 33.7). All other independent variables, such as diabetes and hypertension, did not show a significant association with the POR for a reduced eGFR.

Table 7. Prevalence odds of a reduced eGFR in relation to cadmium and β_2M excretion rates normalized to C_{cr}.

Independent Variables/ Factors	Reduced eGFR [a]				
	Number of Subjects	POR	95% CI		*p*
			Lower	Upper	
Age, years	448	1.140	1.072	1.211	<0.001
BMI, kg/m^2	448	1.066	0.950	1.198	0.278
Log[$(E_{Cd}/C_{cr}) \times 10^5$], µg/L filtrate	448	2.251	1.043	4.858	0.039
Gender (F/M)	334/114	0.674	0.176	2.583	0.565
Smoking	140	0.885	0.270	2.899	0.840
Diabetes	69	1.216	0.394	3.753	0.734
Hypertension	218	1.199	0.487	2.957	0.693
$(E_{\beta 2M}/C_{cr}) \times 100$, µg/L filtrate					
<300	184	Referent			
300–999	156	8.310	2.655	26.01	<0.001
≥1000	108	33.731	4.193	271.3	0.001

[a] Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73 m^2; POR, prevalence odds ratio; CI, confidence interval. Data were generated from multivariable logistic regression analyses, relating the POR for a reduced eGFR to eight independent variables (first column). *p*-values < 0.05 indicate a statistically significant increase in the POR for a reduced eGFR.

An equivalent logistic regression was conducted using E_{cr}-normalized E_{Cd} and $E_{\beta 2M}$ data (Table 8).

Table 8. Prevalence odds of a reduced eGFR in relation to cadmium and β_2M excretion rates normalized to Ecr.

Independent Variables/ Factors	Reduced eGFR [a]				
	Number of Subjects	POR	95% CI		*p*
			Lower	Upper	
Age, years	448	1.101	1.045	1.160	<0.001
BMI, kg/m^2	448	1.032	0.929	1.147	0.555
Log[(E_{Cd}/E_{cr}) × 10^3], µg/g creatinine	448	1.278	0.630	2.593	0.497
Gender (F/M)	334/114	0.671	0.181	2.484	0.550
Smoking	140	0.762	0.240	2.418	0.644
Diabetes	69	1.614	0.581	4.484	0.359
Hypertension	218	1.041	0.449	2.415	0.926
($E_{\beta 2M}/C_{cr}$) × 100, µg/g creatinine					
<300	160				
300–999	155	3.204	1.226	8.375	0.018
≥1000	133	19.042	2.387	151.907	0.005

[a] Reduced eGFR is defined as the estimated glomerular filtration rate ≤ 60 mL/min/1.73 m^2; POR, prevalence odds ratio; CI, confidence interval. Data were generated from multivariable logistic regression analyses, relating the POR for a reduced eGFR to eight independent variables (first column). *p*-values < 0.05 indicate a statistically significant increase in the POR for a reduced eGFR.

The POR values for a reduced eGFR rose with age (POR = 1.10), the severity of tubular dysfunction, $E_{\beta 2M}/E_{cr}$ 300–999 µg/g creatinine (POR = 3.20), and $E_{\beta 2M}/E_{cr}$ ≥ 100 µg/g creatinine (POR = 19.0). Associations of POR for a reduced eGFR with E_{Cd}/E_{cr} and all other variables were statistically insignificant.

4. Discussion

This study used a cross-sectional analysis of kidney dysfunction, tubular proteinuria, and eGFR decline to determine the differential impact of Cd exposure in men and women. Whereas many previous studies focused primarily on Cd-induced tubulopathy in women, we investigated these health outcomes in both men and women along with confounding risk factors, smoking, diabetes, and hypertension. The excretion rate of Cd and β_2M (E_{Cd} and $E_{\beta 2M}$) were normalized to the surrogate measure of the GFR, creatinine clearance (C_{cr}). This C_{cr}-normalization of E_{Cd} and $E_{\beta 2M}$ as E_{Cd}/C_{cr} and $E_{\beta 2M}/C_{cr}$ depicts the excretion rates per functional nephron; thereby, it corrects for differences in the number of functioning nephrons among the study subjects [7]. This C_{cr}-normalized excretion rate also corrects for urine dilution, but it is unaffected by creatinine excretion (E_{cr}). Thus, E_{Cd}/C_{cr} and $E_{\beta 2M}/C_{cr}$ provide an accurate quantification of the kidney burden of Cd and its toxicity to kidney tubular cells.

We selected subjects from two population-based studies, undertaken in an area with endemic Cd contamination in the Mae Sot district, Tak province [8], and in a control, non-contaminated area in the Nakhon-Si-Thammarat province of Thailand [9,10]. The Cd content of the paddy soil samples from the Mae Sot district exceeded the standard of 0.15 mg/kg, and the rice samples collected from households contained four times the amount of the permissible Cd level of 0.1 mg/kg [16].

4.1. Exposure Levels of Cadmium in Women Versus Men

Men and women in this cohort carry the same body burden of Cd, which is evident from a nearly identical mean (E_{Cd}/C_{cr}) × 100 values of 3.12 vs. 3.21 µg/L filtrate. The sources of Cd could be differentiated through an analysis of blood–urine Cd relationships.

$[Cd]_b$ and E_{Cd}/C_{cr} correlated strongly with each other in women (R^2 = 0.624) and men (R^2 = 0.661) (Figure 1a), and the covariate-adjusted means $[Cd]_b$ showed a stepwise increase through the E_{Cd}/C_{cr} tertiles in both genders. Notably, E_{Cd}/C_{cr} explained a larger fraction of the variation in $[Cd]_b$ in men than it did in women (η^2 0.407 vs. 0.105) (Figure 1b). The variability in $[Cd]_b$ was associated mostly with E_{Cd}/C_{cr} in both genders, while smoking explained a larger fraction of the $[Cd]_b$ variability among men than among women (5.5% vs.

2.8%). This result was expected, given the high % of smokers in the male group (68.4% vs. 18.6%) and the higher mean $[Cd]_b$ in men than in women (3.25 vs. 4.36 μg/L). In men only, the $[Cd]_b$ variation was associated with diabetes.

4.2. The Toxic Manifestation of Cadmium Exposure in Women Versus Men

An independent health survey reported that the prevalence of chronic kidney disease (CKD), defined as the eGFR ≤ 60 mL/min/1.73 m^2, among Mae Sot residents was 16.1%, while the prevalence of tubulopathy, referred to as tubular proteinuria, was 36.1% [17]. This reported tubular proteinuria was based on the cut-off value of $E_{\beta 2M}/E_{cr}$ at 300 μg/g creatinine [6], which equates to $E_{\beta 2M}/C_{cr}$ of 2–3 μg/ L filtrate or $(E_{\beta 2M}/C_{cr}) \times 100$ of 200–300 μg/ L filtrate. Notably, the cut-off value for $E_{\beta 2M}/E_{cr}$ at 300 μg/g creatinine was used as the critical effect of exposure to Cd in the human diet [6].

In this cohort, tubular proteinuria affected more than half of women (61.1%) and men (52.6%). One of four women had severe tubular impairment [$(E_{\beta 2M}/C_{cr}) \times 100 \geq 1000$ μg/L filtrate], whereas one of five men had this abnormality.

In women, $E_{\beta 2M}/C_{cr}$ showed a moderate positive association with age (β = 0.131), and an equally strong association with E_{Cd}/C_{cr} (β = 0.306) and diabetes (β = 0.349) (Table 3). In men, $E_{\beta 2M}/C_{cr}$ did not show a significant association with age or E_{Cd}/C_{cr}, but this tubular defect was associated with diabetes only (β = 0.279). In the covariance analysis, the contribution of E_{Cd}/C_{cr} to the variability of $E_{\beta 2M}/C_{cr}$ in women was demonstrable together with a dose–effect relationship after the adjustment of the covariates and interactions (Figure 2d). In contrast, the contribution of E_{Cd}/C_{cr} to the variation of $E_{\beta 2M}/C_{cr}$ in men was statistically insignificant (Figure 2d). An association of the marker of tubular dysfunction ($E_{\beta M}$) and diabetes seen in both men and women is in line with the current knowledge that diabetes adversely affects both glomerular (GFR) and tubular function, termed diabetic tubulopathy [18,19].

The overall mean eGFR was 90 mL/min/1.73 m^2, and the overall prevalence of eGFR ≤ 60 mL/min/1.73 m^2 in this cohort was 6.9% (Table 1). The difference in the % of the reduced eGFR in women and men (8.1% vs. 3.5%) did not reach a statistical significance level (p = 0.097), nor did the difference in the mean eGFR in women and men (p = 0.145). The weaker effect of Cd exposure on the eGFR in men, compared to women, remains to be confirmed with a sufficiently large sample group of men. However, the regression analysis also indicated gender differences in susceptibility to the nephrotoxicity of Cd (Table 5). In women, the eGFR was inversely associated with age (β = −0.511), E_{Cd}/C_{cr} (β = −0.185), and diabetes (β = −0.128). In comparison, the eGFR in men was not associated with E_{Cd}/C_{cr}, while showing an inverse association with age (β = −0.506) and hypertension (β = −0.212). Adverse effects of hypertension and diabetes on the eGFR have been noted in a cross-sectional study of the general U.S. population, where a Cd-induced GFR reduction was more pronounced in those who had diabetes and/or hypertension [20].

We speculate that gender differences in the levels of some protective factors, notably body status of nutritionally essential metals such as iron and zinc, may contribute to the increased susceptibility to Cd nephrotoxicity that was seen in women. Similarly, environmental Cd exposure has been linked to a reduction in the eGFR among participants in various cycles of the U.S. National Health and Nutrition Examination Survey (NHANES) undertaken over 18 years (1999 to 2016) [20–22]. Lin et al. (2014) reported that the risk for a reduced eGFR was higher in those with lower serum zinc (OR 3.38) compared to those with similar Cd exposure levels and serum zinc > 74 μg/dL (OR 2.04) [22].

4.3. Increment of β_2M Excretion as GFR Falls

The protein β_2M with the molecular weight of 11,800 Da is filtered freely by the glomeruli and is reabsorbed almost completely by the kidney's tubular epithelial cells [13]. Thus, the defective tubular re-absorption of β_2M will result in an enhanced excretion rate of β_2M [23–26]. The loss of nephrons also raises the excretion of β_2M for the following reasons [23,26]. When the reabsorption rate of β_2M per nephron remains constant, its

excretion will vary directly with its production. If the production and reabsorption per nephron remain constant as nephrons are lost, the excretion of β_2M will rise [27].

It can thus be expected that the excretion of β_2M will increase when the GFR falls for any causes. Indeed, $E_{\beta 2M}/C_{cr}$ was inversely associated with the eGFR in both women and men (Figure 3c), although the causes of their eGFR decreases seemed to be different. In a quantitative analysis (Figure 3d), the η^2 value describing the effect size of $E_{\beta 2M}/C_{cr}$ on the eGFR variability was 1.7-fold larger in women than in men (0.114 vs. 0.066). In both women and men, the eGFR was the lowest in those who had $(E_{\beta 2M}/C_{cr}) \times 100 \geq 1000$ μg/L filtrate, indicative of severely impaired tubular function.

Notably, the POR for a reduced eGFR was increased by 8.3-fold and 33.7-fold in those with $(E_{\beta 2M}/C_{cr}) \times 100$ of 300–999 and ≥1000 μg/L filtrate, respectively (Table 7), compared to those with $(E_{\beta 2M}/C_{cr}) \times 100 < 300$ μg/L filtrate. A substantial loss of nephron function was a likely cause of the massive increases in $E_{\beta 2M}/C_{cr}$ that was seen in those who had an eGFR below 60 mL/min/1.73 m^2.

4.4. The Pitfall of Adjusting Excretion Rate to Ecr and Implication for Health Risk Estimation

The C_{cr}-normalized data indicate that women and men shared the same burden of Cd (Table 1). The data also indicate that the % of women and men across the three categories of tubulopathy were all statistically different, thereby linking Cd exposure to the severity of tubulopathy in both genders. The logistic regression data (Table 7) show that the likelihood of having a reduced eGFR was increased by 8.3-fold and 33.7-fold in those who had $(E_{\beta 2M}/C_{cr}) \times 100$ of 300–999 and ≥1000 μg/L filtrate compared to those with $(E_{\beta 2M}/C_{cr}) \times 100 < 300$ μg/L filtrate.

The E_{cr}-normalized data indicated that the mean E_{Cd}/E_{cr} in women was statistically higher than that of men (4.26 vs. 3.30 μg/g creatinine). They also indicated that the difference in % of women across the three tubulopathy categories was minuscule, and that the % distribution of men across the tubulopathy categories was statistically significant. These data suggest an association of Cd exposure and the severity of tubulopathy in men only. The logistic regression data (Table 8) show that the likelihood of having a reduced eGFR was increased by 3.2-fold and 19-fold in those who had $E_{\beta 2M}/E_{cr}$ of 300–999 and ≥1000 μg/g creatinine compared to those with $E_{\beta 2M}/E_{cr} < E_{\beta 2M}/E_{cr}$.

Previously, $E_{\beta 2M}/E_{cr}$ of 100–299, 300–999, and ≥1000 μg/g creatinine were found to be associated with 4.7-fold, 6.2-fold, and 10.5-fold increases in the prevalence odds of a reduced eGFR [28,29]. Similarly, a rise in $E_{\beta 2M}/E_{cr}$ to levels not higher than 100 μg/g creatinine was associated with an increased risk of hypertension in the general Japanese population [30], while the prospective cohort data showed that $E_{\beta 2M}/E_{cr}$ was associated with a 79% increase in the likelihood of having a large decline in the eGFR (10 mL/min/1.73 m^2) over a five-year period [31]. Thus, a cut-off value for $E_{\beta 2M}/E_{cr}$ above 300 μg/g creatinine does not reflect an early warning sign of the nephrotoxicity of Cd. The utility of this $E_{\beta 2M}/E_{cr}$ value as a toxicity criterion to derive a toxicity threshold level for Cd is inappropriate.

In summary, adjusting E_{Cd} and $E_{\beta 2M}$ to E_{cr} produces an erroneous interpretation of the effect of Cd exposure on the eGFR, while underestimating the severity of Cd-induced tubulopathy, especially among women. These data call into question the utility of $E_{\beta 2M}/E_{cr}$ of 300 μg/g creatinine to represent the critical effect of exposure to Cd in the human diet. New health guidance values need to be established for this toxic metal, and new public measures are needed to minimize the Cd contamination of food chains.

4.5. Strength and Limitation

In this cohort, the levels of environmental exposure among the participants were assessed by measuring the blood Cd and urinary Cd excretion rates. Strong correlations between these two parameters were seen in both men and women (Figure 1). Both the tubular and glomerular function were examined concurrently together with confounding factors, smoking, hypertension, and type 2 diabetes. These are the strengths of our study.

The small number of males from high- (n = 84) and low-exposure (n = 30) locations is a limitation. This precludes an analysis of both genders separately from both locations that may help to rule out any other environmental effects on adverse kidney outcomes in women. In addition, the heterogeneity in the hormonal status, notably estrogen, in female participants who are menopausal and post-menopausal is a limitation [32].

5. Conclusions

The excretion of β_2M above 300 μg/g creatinine (≈2–3 μg/L filtrate) and a reduction in the glomerular function, indicated by an eGFR below 60 mL/min/1.73 m^2, are the manifestations of severe kidney toxicities due to chronic exposure to Cd that are more prevalent and more severe in women than men of the same body burden.

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Informed Consent Statement: Informed consent was obtained from all participants in the study prior to their participation.

Data Availability Statement: All data are contained within this article.

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