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Albuminuria in People Chronically Exposed to Low-Dose Cadmium Is Due to Rising Blood Pressure Levels

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Abstract: Exposure to low-dose environmental pollutant cadmium (Cd) increases the risks of both albuminuria and hypertension by mechanisms which are poorly understood. Here, multiple-regression and mediation analyses were applied to data from 641 Thai subjects of whom 39.8%, 16.5%, 10.8%, and 4.8% had hypertension, albuminuria, diabetes, and chronic kidney disease (CKD), defined as the estimated glomerular filtration rate (eGFR) ≤ 60 mL/min/1.73 m^2, respectively. To correct for interindividual differences in urine dilution and surviving nephrons, the excretion rates of Cd (E_{Cd}), albumin (E_{alb}) and β$_2$-microglobulin ($E_{\beta 2M}$) were normalized to creatinine clearance (C_{cr}) as E_{Cd}/C_{cr}, E_{alb}/C_{cr} and $E_{\beta 2M}/C_{cr}$. Respective risks of having CKD and hypertension rose to 3.52 (95%CI: 1.75, 7.05) and 1.22 (95% CI:1.12, 1.3) per doubling Cd body burden. Respective risk of having albuminuria increased 2.95-fold ($p = 0.042$), and 4.17-fold ($p = 0.020$) in subjects who had hypertension plus severe and extremely severe tubular dysfunction, defined according to elevated β$_2$M excretion rates. In multiple regression analysis, E_{alb}/C_{cr} increased linearly with both systolic blood pressure (SBP, β = 0.263) and diastolic blood pressure (DBP, β = 0.150), while showing an inverse association with eGFR (β = −0.180). The mediation model analyses inferred that a declining eGFR induced by Cd contributed to 80.6% of SBP increment ($p = 0.005$), which then fully mediated an elevation of albumin excretion ($p < 0.001$). The present study provides, for the first time, evidence that causally links Cd-induced eGFR reductions to blood pressure elevations which enhance albumin excretion.

Keywords: albuminuria; blood pressure; β$_2$-microglobulin; cadmium; estimated glomerular filtration rate; mediation analysis

1. Introduction

An approximate of 8-13% of the world's population is living with chronic kidney disease (CKD) [1–3]. In early stages, CKD is asymptomatic, and it is diagnosed when there is a substantial loss of functioning nephrons, evident from a fall of the estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 (termed low eGFR) [1–3]. This CKD diagnostic stage often co-exists with disease comorbidities such as hypertension and proteinuria [2]. Alarmingly, CKD is predicted to become the fifth leading cause of years of life lost by 2040 [4,5].

Albuminuria is designated, when the excretion of albumin (E_{alb}), typically measured as the albumin-to-creatinine ratio (ACR), rises to levels above 20 and 30 mg/g creatinine in men and women,

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respectively [1–3]. Albuminuria that persists for at least 3 months is also employed as a CKD diagnostic criterion [1–3].

Elevated risks of kidney damage [6–8], albuminuria [9–11], proteinuria [12,13] and CKD [14–17] have repeatedly been linked to chronic exposure to the metal pollutant cadmium (Cd) in many countries. There is also an increased mortality risk among CKD patients who had an elevated Cd body burden, reflected by Cd excretion rates ≥ 0.60 μg/g creatinine [18].

Cd is a metal contaminant with no nutritional value or physiological role, and it presents worldwide public health concerns because it is highly toxic [19]. For most people, exposure to Cd is unavoidable because it is found in most food types [20–22], cigarette smoke and polluted air [23–25]. Most acquired Cd accumulates within the kidney tubular cells, where its levels increase through to the age of 50 years but decline thereafter due to its release into the urine as the injured tubular cells die [19,26]. Because most or all excreted Cd emanates from injured or dying tubular cells, excretion of Cd reflects the injury at the present time, not the risk of injury in the future [25,26].

The most frequently reported effects of environmental Cd exposure include kidney tubular cell damage and tubular dysfunction, indicated by an increased excretion of the low-molecular weight protein, β_2-microglobulin (β_2M) [25]. An increase β_2M excretion above 300 μg/g creatinine was used in the toxicological risk assessment of Cd in the human diet [27,28]. Current evidence has implicated the circulating β_2M in blood pressure regulation [29], and β_2M excretion above 300 μg/g creatinine is indicative of enhanced increased risk of hypertension and severe kidney pathologies, such as rapid kidney functional deterioration and nephron loss for any reason [30–32].

The present study has three major objectives. The first is to examine the dose-response relationship between environmental Cd exposure levels, and three adverse outcomes of such exposure, namely CKD, hypertension, and defective tubular function. Excretion of Cd (E_{Cd}) and β_2M ($E_{\beta 2M}$) were used as indicators of long-term exposure or body burden of Cd and tubular dysfunction, respectively [25]. The second objective is to explore a connection between E_{alb} and the rising levels of systolic and diastolic blood pressure (SBP and DBP) in Cd-exposed people. The third objective is to address female preponderance effects of environmental Cd exposure on blood pressure. These study objectives are formulated based on current stage of knowledge on the epidemiology of Cd toxicity and the significance of albuminuria as an independent risk factor for hypertension [33], a strong independent risk factor for worse outcomes of cardiovascular disease, incident CKD and its progression to kidney failure, especially among diabetics [34–37].

2. Materials and Methods

2.1. Participant Selection

We assembled archived data from large Thai population-based cohorts of residents in the Mae Sot District, Tak Province, where environmental Cd contamination was endemic (n = 310), and two low-exposure areas in Bangkok (n = 192) and Nakhon-Si-Thammarat Province (n = 139).

The Institutional Ethical Committees of Chulalongkorn University, Chiang Mai University and the Mae Sot Hospital approved the study protocol for the Mae Sot and Bangkok groups [38]. The Human Research Ethics Committee of Walailak University approved the study protocol for the Nakhon Si Thammarat group [39]. All participants gave informed consent prior to participation.

For all groups, exclusion criteria were pregnancy, breast-feeding, a history of metal work, and a hospital record or physician's diagnosis of an advanced chronic disease.

For the low-exposure groups, those aged 19 years or older were selected. The health status was ascertained by physician's examination reports and routine blood and urinary chemistry profiles. For the Mae Sot group, those who had resided at their current addresses for 30 years or longer were selected. The sociodemographic data, educational attainment, occupation, health status, family history of diabetes, and smoking status were obtained by structured interview questionnaires.

The diagnosis of hypertension relied primarily on the assessment made by the presiding physician and the recorded use of anti-hypertensive medication. Of 255 participants with

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hypertension, 242 (94.9%) were being treated, and 13 hypertensive cases were identified during our visit.

2.2. Assessment of Cadmium Exposure Levels and Its Effects

Samples of urine, whole blood, and plasma were collected from all participants after an overnight fast, and were stored at -80 ºC for later analysis. Plasma samples were assayed for the concentration of creatinine, while urine samples were assayed for the concentrations of creatinine, Cd, β_2M and alb, detailed in previously [38,39].

The eGFR was computed with equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) [40–42]. CKD stages 1, 2, 3, 4, and 5 corresponded to eGFR of 90–119, 60–89, 30–59, 15–29, and < 15 mL/min/1.73 m^2, respectively [40,41].

2.3. Normalization of Cadmium, β2M and Albumin Excretion Rates

The excretion of x (E_x) was normalized to creatinine clearance (C_{cr}) as $E_x/C_{cr} = [Cd]_u[cr]_p/[cr]_u$, where x = Cd, β_2M or alb, $[x]u$ = 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 an amount of x excreted per volume of the glomerular filtrate [43]. This C_{cr}-normalization corrects for urine dilution and the number of functioning nephrons simultaneously, and it is not influenced by muscle mass.

The excretion of x (E_x) was normalized to E_{cr} as $[x]_u/[cr]_u$, where x= Cd, β_2M or alb, $[x]_u$ = urine concentration of x (mass/volume), and $[cr]_u$ = urine creatinine concentration (mg/dL). E_x/E_{cr} was expressed as an amount of x excreted per g of creatinine. This E_{cr}-normalization corrects for urine dilution only. This method of normalization of excretion rate is affected by interindividual differences in muscle mass which produces non-differential errors and a clear dose-response relationship of E_{Cd} and E_{alb} cannot be established [44,45].

2.4. Mediation Analysis for Cause-Effect Inference

A simple mediation model with a single mediator (M) and the Sobel test for the statistical significance of an indirect effect of the independent variable X were as described by MacKinnon et al. (1995) and Preacher and Hayes (2004) [46–48].

A mediation model with M as a mediator of the effect of the independent variable X on the dependent variable Y, and standardized β coefficients describing the relationships of X, M, and Y are depicted below.

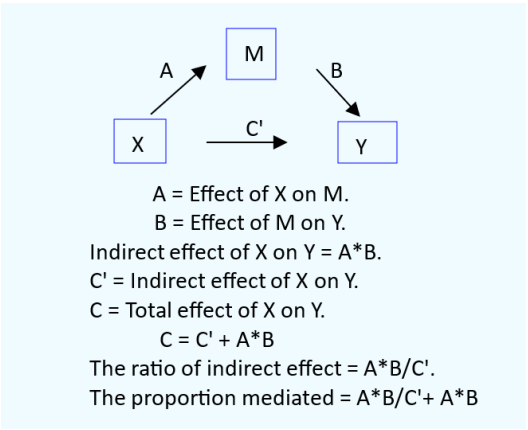

Diagram 1. A mediation model with a single mediator.

2.5. Statistical Analysis.

Data were analyzed using IBM SPSS Statistics 21 (IBM Inc., New York, NY, USA). To assess mean differences across Eβ_2M groups, the Kruskal-Wallis Test was used. The Pearson chi-squared

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test was used to assess differences in percentages and prevalences of smoking, hypertension, low eGFR, diabetes, and albuminuria. The one-sample Kolmogorov–Smirnov test was used to ascertain the conformity to a normal distribution of continuous variables. Logarithmic transformation was applied to E_{Cd}, $E_{\beta 2M}$ and E_{alb}, which showed a right-skewed distribution. For eGFR, no data transformation was required because the distribution of eGFR values was left-skewed. Multiple linear regression was conducted to E_{alb} predictors.

Logistic regression was conducted to evaluate the effects of Cd exposure and other independent variables on the prevalence odds ratio (POR) for CKD, hypertension, tubular dysfunction, and albuminuria. All reported POR values were adjusted for potential confounders.

Univariate analysis with Bonferroni correction in multiple comparisons was used to obtain covariate adjusted mean E_{alb}/C_{cr} and eta square (η^2). For all tests, *p*-values ≤ 0.05 were considered to indicate statistical significance.

3. Results

3.1. Cohort Participants

Characteristics of 641 cohort participants can be found in Table 1.

Table 1. Descriptive characteristics of cohort participants according to β2-microglobulin excretion rates.

Parameters	All *n* = 641	$E_{\beta 2M}/C_{cr}$, µg/L filtrate			
		< 1.0, *n* = 442	1.0 – 2.9, *n* = 69	3.0 – 9.9, *n* = 61	≥ 10, *n* = 61
Age, years	47.5 (10.6)	44.2 (8.9)	51.1 (9.1)	55.5 (10.9)	58.4 (9.8) ***
Age range, years	16 – 80	16 – 69	21 – 75	31 – 80	42 – 79
BMI, kg/m²	24.4 (4.0)	24.2 (3.7)	24.7 (5.2)	24.8 (4.1)	24.8 (4.2)
% BMI ≥ 30 kg/m² (obese)	6.3	5.0	10.4	7.2	10.0
eGFR [a], mL/min/1.73 m²	95 (19)	101 (15)	93 (14)	83 (16)	67 (18) ***
% Low eGFR (CKD)	4.8	0.7	1.4	5.8	37.7 ***
% Women	66.9	63.8	71.1	72.5	78.7
% Hypertension	39.8	34.4	42.2	52.2	62.3 ***
% Smoking	29.0	31.0	30.4	15.9	27.9
% Diabetes	10.8	0.9	14.7	29.0	54.7 ***
Systolic blood pressure, mmHg	126 (16)	122 (13)	129 (16)	135 (18)	140 (18) ***
Diastolic blood pressure, mmHg	80 (10)	78.4 (9.2)	82.8 (10.6)	84 (10)	84 (9) ***
Normalized to E_{cr} (E_x/E_{cr})					
E_{Cd}/E_{cr}, µg/g creatinine	2.98 (4.01)	3.00 (3.73)	3.43 (4.35)	2.35 (4.24)	3.06 (5.17) ***
$E_{\beta 2M}/E_{cr}$, µg/g creatinine	516 (2153)	37 (36)	221 (74)	697 (274)	4124 (5854) ***
E_{alb}/E_{cr}, mg/g creatinine (ACR)	22 (65)	12 (31)	14 (25)	22 (51)	74 (147) ***
% Albuminuria [b]	14.9	9.4	13.3	17,2	38.3 ***
Normalized to C_{cr}, (E_x/C_{cr}) [c]					
(E_{Cd}/C_{cr}) ×100, µg/L filtrate	2.37 (3.35)	2.27 (2.84)	2.67 (3.84)	2.01 (3.52)	3.17 (5.34) **
($E_{\beta 2M}/C_{cr}$) ×100, µg/L filtrate	556 (2857)	27 (26)	170 (52)	587 (204)	4789 (8159) ***
(E_{alb}/C_{cr}) ×100, µg/L filtrate	22 (79)	9 (25)	11 (22)	20 (50)	88 (191) ***
% (E_{alb}/C_{cr}) ×100 ≥ 20 µg/L filtrate	16.5	9.8	11.7	21.9	45.0 ***

n, number of subjects; BMI, body mass index; eGFR, estimated glomerular filtration rate; cr, creatinine; alb, albumin; Cd, cadmium; ACR, albumin-to-creatinine ratio. [a] eGFR was determined using equations of the Chronic Kidney Disease Epidemiology Collaboration (CKD−EPI). [b] Albuminuria was defied as ACR ≥ 20 and ≥

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30 mg/g creatinine in men and women, respectively. [c] $E_{Cd}/C_{cr} = [Cd]_u[cr]_p/[cr]_u$. Data for BMI and urinary albumin were from 617, and 451 subjects, respectively. All other data were from 641 subjects. Continuous variables are expressed as arithmetic mean and standard deviation (SD) values. For all tests, $p \leq 0.05$ identifies statistical significance, determined with the Pearson Chi-Square test for differences in percentages and the Kruskal-Wallis test for differences of means among $E_{\beta 2M}/Ccr$ groups. ***$p < 0.001$; **$p = 0.003$.

The overall mean age of cohort participants was 47.5 years and the overall mean arithmetic (geometric) mean for Cd excretion rate was 0.024 (0.009) µg/L filtrate, corresponding to 2.98 (1.11) µg/g creatinine. The % of smokers and diabetics were 29 and 10.8, respectively. Hypertension was the most prevalent (39.8%) followed by albuminuria (16.5%), and low eGFR (4.8%).

To explore potential effects of tubular dysfunction, participants were grouped by $E_{\beta 2M}/C_{cr}$ values, and 422, 69, 61 and 61 participants were found to have $E_{\beta 2M}/C_{cr}$ values of 1.0 − 2.9, 3.0 − 9.9 and ≥ 10 µg/L filtrate, respectively. The % hypertension, albuminuria and low eGFR rose across $E_{\beta 2M}/C_{cr}$ groups. In the highest $E_{\beta 2M}/C_{cr}$ group, % hypertension, albuminuria and a low eGFR were 62.3, 38.3 and 37.7 respectively. The corresponding % figures in the lowest $E_{\beta 2M}/C_{cr}$ group were 34.4, 9.4 and 0.7. Mean SBP and mean DBP increased across $E_{\beta 2M}/C_{cr}$ groups.

3.2. Effects of Cadmium on the Risks of Having CKD, Hypertension and Tubular Defect

Table 2 provides results of three logistic regression models for CKD, hypertension, and tubular dysfunction.

Table 2. Prevalence odds ratios for three adverse outcomes in relation to cadmium body burden and other independent variables.

Independent Variables/Factors	CKD [a]		Hypertension		Tubular dysfunction [c]	
	POR (95% CI)	*p*	POR (95% CI)	*p*	POR (95% CI)	*p*
Age, years	1.156 (1.096, 1.218)	<0.001	1.059 (1.041, 1.078)	<0.001	1.112 (1.075, 1.149)	<0.001
BMI, kg/m²	1.027 (0.930, 1.135)	0.599	1.120 (1.071, 1.171)	<0.001	0.962 (0.898, 1.031)	0.273
$Log_2[(E_{Cd}/C_{cr}) \times 10^5]$, µg/L filrate	3.517 (1.754, 7.051)	<0.001	1.218 (1.124, 1.319)	<0.001	1.037 (0.917, 1.173)	0.567
Gender	0.532 (0.150, 1.887)	0.329	1.462 (0.964, 2.215)	0.074	1.321 (0.617, 2.571)	0.413
Smoking	0.708 (0.226, 2.215)	0.553	1.477 (0.959, 2.274)	0.077	1.163 (0.585, 2.315)	0.667
Diabetes	4.839 (1.725, 13.58)	0.003	2.008 (1.072, 3.726)	0.030	16.12 (7.219, 36.02)	<0.001
Hypertension	1.152 (0.497, 2.669)	0.742	–	–	1.556 (0.924, 2.622)	0.097
CKD [b]	–	–	0.877 (0.393, 1.958)	0.750	17.67 (5.155, 60.56)	<0.001

CKD, chronic kidney disease; POR, prevalence odds ratio; CI, confidence interval; BMI, body mass index. [a] CKD was defined as eGFR ≤ 60 mL/min/1.73 m². [b] Tubular dysfunction was indicated by $E_{\beta 2M}/C_{cr} \geq 3.0$ µg/L filtrate. For all tests, *p*-values ≤ 0.05 indicate a statistically significant association between an individual independent variable and the POR for CKD, hypertension, or tubular proteinuria.

The risks of having CKD, hypertension and tubular dysfunction were not affected by gender or smoking, but age, BMI and diabetes did. Age increased the risk of all three outcomes, while a rise in BMI increased the risk of hypertension only. The risks of having CKD and hypertension rose 3.5-fold ($p < 0.001$) and 1.2-fold (p <0.001), respectively, as the body burden of Cd increased two-fold.

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Doubling Cd burden had little effect on the risk of having tubular dysfunction, defined as $E_{\beta 2M}/C_{cr}$ rates ≥ 3.0 μg/L filtrate [POR = 1.037 (95%CI: 0.917, 1.173), p = 0.567].

Tubular dysfunction was 17.7-time more prevalent among those with CKD. In comparison, all three outcomes were more prevalent among diabetics, compared with non-diabetics with the same overall Cd body burden.

3.3. Effects of Hypertension on the Prevalence of Albuminuria

Table 3 provides results of logistic regression models for albuminuria prevalence in all subjects and subgroups with or without hypertension.

Table 3. Hypertension as the key determinant of albuminuria.

Independent Variables/Factors	Albuminuria [a]					
	All, n = 445		Normotension, n = 229		Hypertension, n = 216	
	POR (95% CI)	p	POR (95% CI)	p	POR (95% CI)	p
Age, years	1.000 (0.962, 1.039)	0.988	1.004 (0.942, 1.069)	0.913	0.991 (0.940, 1.045)	0.739
$Log_2[(E_{Cd}C_{cr})\times 10^5]$, μg/L filtrate	1.042 (0.905, 1.199)	0.571	0.940 (0.750, 1.179)	0.593	1.088 (0.894, 1.325)	0.401
Obese	1.346 (0.525, 3.453)	0.536	1.545 (0.277, 8.626)	0.620	0.930 (0.269, 3.215)	0.909
CKD	3.312 (1.272, 8.623)	0.014	3.766 (0.626, 22.65)	0.147	4.293 (1.072, 17.19)	0.040
Diabetes	3.603 (1.559, 8.326)	0.003	1.913 (0.397, 9.216)	0.419	5.376 (1.795, 16.10)	0.003
Gender	1.458 (0.715, 2.972)	0.299	1.944 (0.690, 5.475)	0.208	0.869 (0.304, 2.483)	0.793
Smoking	0.835 (0.400, 1.743)	0.630	0.812 (0.274, 2.409)	0.708	0.776 (0.259, 2.328)	0.651
Tubular dysfunction [b]						
Moderate	Referent					
Severe	1.827 (0.822, 4.062)	0.139	0.866 (0.213, 3.517)	0.840	2.946 (1.038, 8.358)	0.042
Extremely severe	2.428 (0.983, 5.997)	0.054	0.655 (0.092, 4.671)	0.673	4.167 (1.250, 13.90)	0.020

POR, prevalence odds ratio; CI, confidence interval; BMI, body mass index. [a] Albuminuria was defined as E_{alb}/C_{cr} ≥ 0.2 mg/L filtrate in both men and women. [b] Moderate, severe, and extremely severe dysfunction were indicated by $E_{\beta 2M}/C_{cr}$ <3, 3.0–9.9 and ≥ 10 μg/L filtrate. For all tests, p-values ≤ 0.05 indicate a statistically significant association between an individual independent variable and the POR for albuminuria.

In an inclusive model, the prevalence of albuminuria was minimally affected by age, Cd body burden, being obese, gender and smoking, while albuminuria was 3.3-time more prevalent in participants with CKD (p = 0.014). Subgroup analysis revealed that hypertension was the key determinant of albuminuria in people with CKD, diabetes, and tubular dysfunction. The prevalence of albuminuria was found to be increased only in those with CKD and hypertension (POR = 4.3, p = 0.040). Similarly, respective prevalences of albuminuria were increased 5.4-fold, 2.9-fold, and 4.2-fold in those with diabetes plus hypertension, severe tubular dysfunction plus hypertension and extremely severe tubular dysfunction plus hypertension.

3.4. Dose-Response Relationship and Quantitative Effect Size

The dose-response relationship of Ealb/Ccr and blood pressure is shown in Figure 1.

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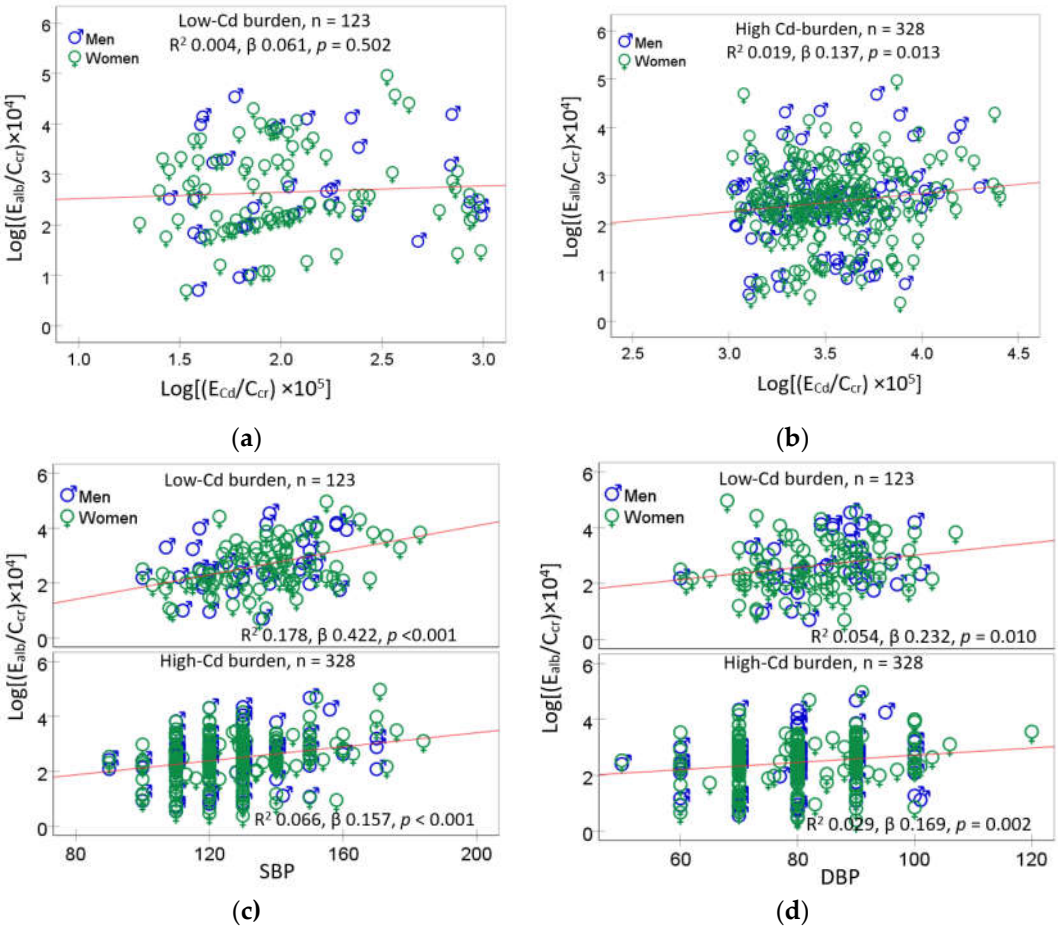

Figure 1. Dose-response relationship of albumin excretion rate, cadmium excretion rate and blood pressure. Scatterplots relate log[(E_{alb}/C_{cr}) × 10^4) to Log[(E_{Cd}/C_{cr}) × 105] in women and men who had low- (**a**) and high-Cd burden (**b**). Scatterplots relate log[(E_{alb}/C_{cr}) × 10^4) to systolic blood pressure (**c**) and diastolic blood pressure (**d**) in women and men with low- or high-Cd burdens. Coefficients of determination (R^2) and p-values and numbers of subjects are provided. The low-, and high-Cd burdens were defined as E_{Cd}/C_{cr} < 0.01 and ≥ 0.01 μg/L filtrate, respectively.

A linear dose-response relationship of E_{alb}/C_{cr} and E_{Cd}/C_{cr} was found only in the high-Cd burden group, defined as E_{Cd}/C_{cr} ≥ 0.01 μg/L filtrate, while a linear dose- response relationship of E_{alb}/C_{cr} and blood pressure measures (SBP and DBP) existed in both low- and high-Cd burden groups. E_{alb}/C_{cr} was more closely associated with SBP than DBP in both Cd burden groups.

Results of multiple linear regression analysis of E_{alb}/C_{cr} can be found in Table 4.

Table 4. Multiple linear regression analysis to define predictors of albumin excretion.

Independent Variables/Factors	Log[(E_{alb}/C_{cr})×10^4], μg/L filtrate					
	All, *n* = 451		Women, *n* = 336		Men, *n* = 115	
	β	*p*	β	*p*	β	*p*
Model 1: SBP						
Age, years	−0.075	0.217	−0.047	0.502	−0.135	0.248
BMI, kg/m²	0.039	0.408	0.062	0.248	−0.042	0.664
eGFR, mL/min/1.73 m²	−0.180	0.001	−0.188	0.002	−0.174	0.090
Log[(E_{Cd}/C_{cr})×10^5], μg/L filtrate	0.103	0.073	0.102	0.132	0.109	0.332
Diabetes	0.179	0.001	0.141	0.023	0.285	0.007
Systolic pressure, mmHg	0.263	<0.001	0.272	<0.001	0.252	0.013
Gender	0.015	0.769	–	–	–	–

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Smoking	0.046	0.379	0.085	0.115	−0.035	0.710
Adjusted R^2	0.147	<0.001	0.143	<0.001	0.146	0.001
Model 2: DBP						
Age, years	−0.006	0.921	0.028	0.695	−0.086	0.465
BMI, kg/m²	0.051	0.289	0.072	0.191	−0.026	0.797
eGFR, mL/min/1.73 m²	−0.195	<0.001	−0.201	0.001	−0.198	0.057
Log[(E_{Cd}/C_{cr})×10⁵], µg/L filtrate	0.122	0.039	0.119	0.085	0.128	0.265
Diabetes	0.220	<0.001	0.189	0.002	0.306	0.005
Diastolic pressure, mmHg	0.150	0.001	0.149	0.005	0.152	0.125
Gender	0.011	0.839	–	–	–	–
Smoking	0.032	0.547	0.068	0.214	−0.040	0.680
Adjusted R^2	0.113	<0.001	0.105	<0.001	0.113	0.006

n, number of subjects; eGFR, estimated glomerular filtration rate; β, standardized regression coefficient; BMI, body mass index; adjusted R^2, coefficient of determination. β indicates strength of association of Ealb/Ccr with eight independent variables (first column). Adjusted R2 indicates the proportion of Ealb/Ccr variation by which all independent variables explained. For all tests, *p*-values ≤ 0.05 indicate a statistically significant association of E_{alb}/C_{cr} with an individual independent variable.

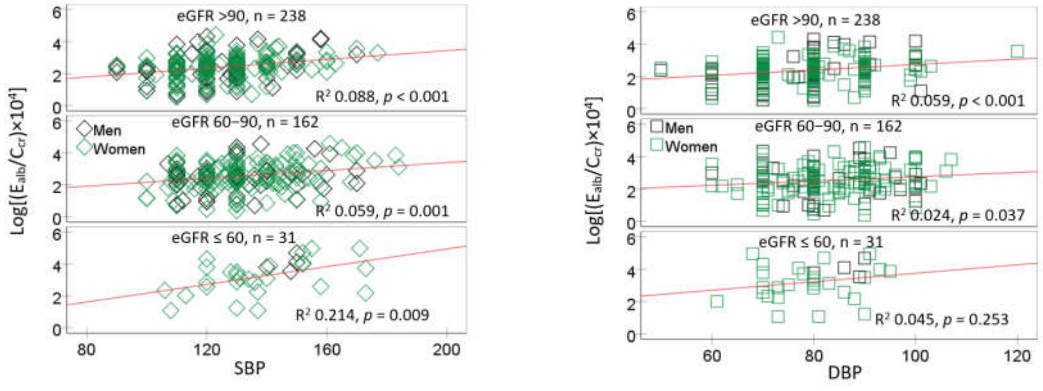
Age, BMI, eGFR, Cd burden, diabetes, blood pressure, gender, and smoking contributed to 14.7% and 11.3% of the total E_{alb}/C_{cr} variation in the inclusive models 1 and 2, respectively.

In the SBP model including all subjects, E_{alb}/C_{cr} varied inversely with eGFR (β = −0.180), while varied directly with SBP (β = 0.263) and diabetes (β = 0.179). In the DBP model, E_{alb}/C_{cr} was inversely associated with eGFR (β = −0.195), while varied directly with DBP (β = 0.150). and diabetes (β = 0.220). In the DBP model only, E_{alb}/C_{cr} showed a positive association with E_{Cd}/C_{cr} (β = 0.122).

In subgroup analysis, E_{alb}/C_{cr} was inversely associated with eGFR only in women, β = −0.188 for SBP model and β = −0.201 for DBP model. An association of Ealb/Ccr with DBP was also found in women only (β =0.149).

E_{alb}/C_{cr} was associated with SBP and diabetes in in both women and men in the SBP model. Similarly, E_{alb}/C_{cr} was associated with diabetes in both women and men in the DBP model.

Figure 2 shows the influences of Cd exposure levels and eGFR levels on albumin excretion among cohort participants.



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

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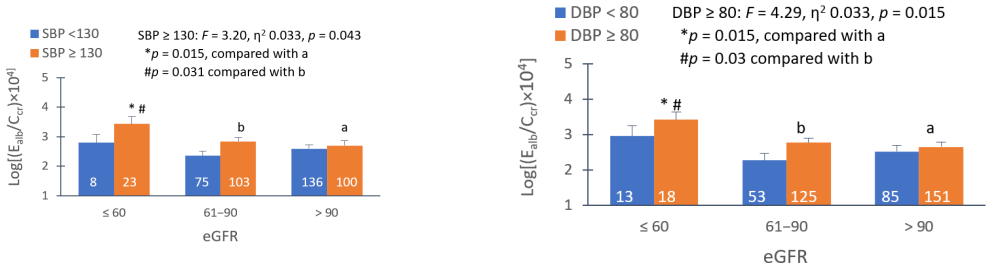


(**c**) (**d**)

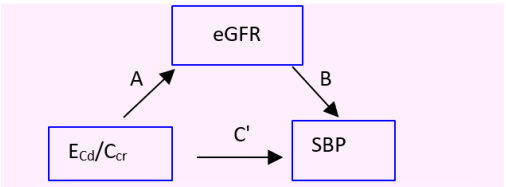
Figure 2. Quantification of effects of blood pressure and eGFR levels on albumin excretion rate. Scatterplots relate log[(E_{alb}/C_{cr}) × 10^4] to SBP (**a**) and DBP (**b**) in subjects with eGFR ≤ 60, 61–90 and ≥ 90 mL/min/1.73 m². Coefficients of determination (R^2) and *p*-values and numbers of subjects are provided. Bar graphs represent mean log[(E_{alb}/C_{cr}) × 10^4] in eGFR subgroups with SBP ≥ 130 mmHg (**c**) and DBP ≥ 80 mmHg (**d**). SBP of 130 mmHg and DBP 80 mmHg were median SBP and DBP values, respectively. All means were adjusted for covariates (age, BMI, E_{Cd}/C_{cr}) and the interaction of smoking × hypertension × diabetes.

E_{alb}/C_{cr} increased linearly with SBP in all three eGFR subgroups (Figure 2a), but the increase of E_{alb}/C_{cr} with DBP was observed only in participants with CKD (Figure 2b). In the univariate analysis for SBP effects with adjustment for covariates (Figure 2c), SBP contributed to 3.3 % of the variation in E_{alb}/C_{cr} in those with SBP higher the median SBP value of 130 mmHg. The mean values for E_{alb}/C_{cr} in participants with high SBP and CKD were 17.2% and 21.2% higher, compared to those with eGFR 61-90 and ≥ 90 mL/min/1.73m², respectively (Figure 2c).

In the univariate analysis for DBP effects with adjustment for covariates (Figure 2d), DBP contributed to 3.3 % of the variation in E_{alb}/C_{cr} in those with DBP higher than the DBP median value of 80 mmHg. The mean values for E_{alb}/C_{cr} in participants with high DBP and CKD were 19.0% and 22.5% higher, compared to those with eGFR 61-90 and ≥ 90 mL/min/1.73m², respectively (Figure 2d).

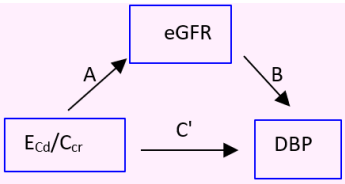
Thus, albumin excretion rate was increased in participants with CKD who also had SBP and DBP higher than 130 and 80 mmHg, respectively.

A simple mediation analysis was conducted to assess potential causal relationships of Cd exposure, eGFR and blood pressure levels (Figure 3).



A = β −0.154, *p* = 0.001
B = β −0.252, *p* < 0.001
C' = β 0.201, *p* < 0.001
C = β −0.256, *p* <0.001
eGFR mediated 80.6% of the SBP increment induced by Cd (*p* = 0.005).

(**a**)



A = β 0.206, *p* < 0.001
B = β −0.093, *p* = 0.052
C' = β −0.132, *p* = 0.006
C = β = −0.152, *p* = 0.001
eGFR mediated insignificant proportion of DBP increment induced by Cd (*p* = 0.080).

(**b**)

The Sobel Test			
SBP	β (SE)	95% CI of β	*p*-value
a	3.799 (1.199)	1.485, 5.974	0.003
b	−0.234 (0.040)	−0.315, −0.157	0.001
*a*b*	−0.889	–	0.005
c	−5.857 (1.090)	−8.072, −3.539	0.001
c'	−4.590 (1.093)	−6.850, −2.404	0.002

SE, standard error of mean; CI, confidence interval. β and 95% CI values were unstandardized, obtained by bootstrapping.

(**c**)

The Sobel Test			
DBP	β (SE)	95% CI of β	*p*-value
a	5.087 (1.203)	2.809, 7.481	0.001
b	−0.052 (0.027)	−0.107, −0.003	0.066
*a*b*	−0.264	–	0.080
c	−2.120 (0.580)	−3.273, −1.014	0.001
c'	−1.835 (0.575)	−3.000, −0.751	0.022

SE, standard error of mean; CI, confidence interval. β and 95% CI values were unstandardized, obtained by bootstrapping.

(**d**)

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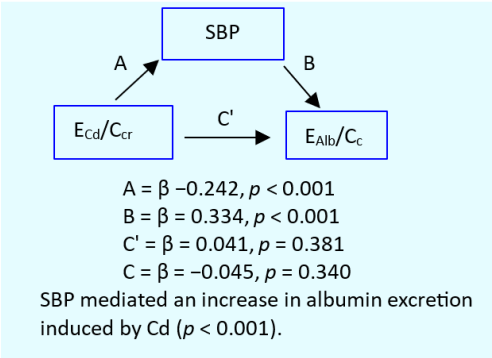
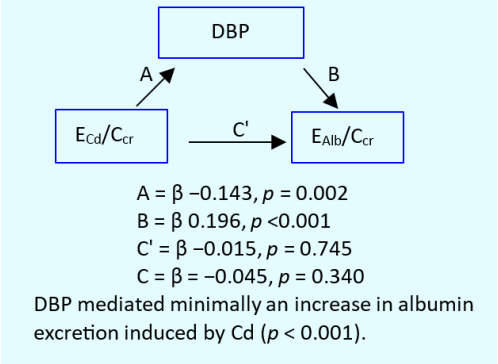
Figure 3. Analysis of eGFR as the mediator of cadmium-induced blood pressure increment. A model depicts eGFR as the mediator of Cd effect on systolic blood pressure (**a**) and diastolic blood pressure (**b**), the Sobel test for an indirect effect of Cd on SBP (**c**) and DBP (**d**).

A simple mediation analysis model of eGFR as the mediator suggested that Cd had direct and indirect effects on SBP and DBP (Figure 3a, 3b). However, the Sobel test results indicated that only the indirect effect of Cd on SBP reached a statistically significant level (Figure 3c,3d). Thus, an increase in SBP was in part due to reductions in eGFR, and the proportion of Cd-induced SBP increment mediated by eGFR was 80.6%. Rising DBP among participants was minimally related to Cd-induced eGFR reductions.

Additionally, two simple mediation models with blood pressure measures (SBP/DBP) as of the mediators were analyzed (Figure 4).



(**a**)



(**b**)

The Sobel Test			
SBP	β (SE)	95% CI of β	*p*-value
a	−5.535 (0.950)	−7.428, −3.550	0.001
b	0.016 (0.002)	0.012, 0.021	0.001
a*b	−0.089	–	<0.001
c	−0.051 (0.056)	−0.155, 0.054	0.384
c'	0.046 (0.055)	0.056, 0.148	0.405

SE, standard error of mean; CI, confidence interval. β and 95% CI values were unstandardized, obtained by bootstrapping.

(**c**)

The Sobel Test			
DBP	β (SE)	95% CI of β	*p*-value
a	−1.999 (0.581)	−3.071, −0.935	0.002
b	0.016 (0.004)	0.008, 0.024	0.001
a*b	−0.032	–	0.009
c	−0.051 (0.053)	−0.155, 0.053	0.340
c'	−0.017 (0.067)	−0.126, 0.093	0.765

SE, standard error of mean; CI, confidence interval. β and 95% CI values were unstandardized, obtained by bootstrapping.

(**d**)

Figure 4. Rising systolic blood pressure as the mediator of an elevation of albumin excretion induced by cadmium. A model depicts SBP (**a**) and DBP (**b**) as the mediator of Cd effect on albumin excretion rate, the Sobel test for an indirect effect of SBP (**c**) and DBP (**d**) on albumin excretion rate.

There was little evidence for a direct effect of Cd on E_{alb}/C_{cr} in SBP (β = 0.041, p = 0.381) and DBP models (β = −0.015, p = 0.745) (Figure 4a,4b). However, Cd had a significant indirect effect on E_{alb}/C_{cr} increment, mediated fully through rising SBP and DBP levels (Figure 4c,4d). Thus, SBP and DBP were the full mediators of an elevation of albumin excretion rate induced by Cd.

4. Discussion

The percentage (%) of hypertension among 641 cohort participants was the highest (39.8) followed by albuminuria (16.5), and low eGFR (4.8), while the percentages of smokers and diabetes were 29 and 10.8, respectively. The % of hypertension and diabetes were in ranges with those reported for the representative U.S. population of 39 and 10.3-13 respectively [49,50]. The CKD (a low eGFR criterion) prevalence in this Thai cohort of 4.8% was lower than 6.8% CKD prevalence figure in Taiwanese population [51], but it was nearly half the prevalence of 9.3% found in a large U.S. population database, where 5175 CKD cases were identified from a total 55,677 U.S. citizens, aged 20–85 years [15].

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4.1. Low eGFR, Hypertension and Albuminuria: Are They Causally Connected?

In the present study, three outcomes of Cd exposure, low eGFR, hypertension and albuminuria were investigated for dose-response and cause-effect relationships. No previous studies have been undertaken to investigate the connection of these outcomes.

Albuminuria was 3.3-time more prevalent among participants with CKD, and the risks of having CKD and hypertension rose, respectively, 3.5-fold and 1.2-fold, when there was a two-fold increase in the body burden of Cd (Tables 1 and 2). Hypertension was found to be the key determinant of albuminuria in participants with CKD; the risk of having albuminuria increased only in those with CKD plus hypertension (POR = 4.3) (Table 3).

We employed two simple mediation models to assess potential causal relationships of Cd exposure, eGFR and blood pressure levels. In the model in which eGFR was the mediator (Figure 3), the increase in SBP levels among cohort participant was in part due to reductions in eGFR. The proportion of Cd-induced SBP increment mediated by eGFR decline was 80.6%. Rising DBP among participants was minimally related to Cd-induced eGFR reductions.

In the model in which SBP or DBP was the mediator (Figure 4), an elevation of albumin excretion rate induced by Cd was through rising SBP and DBP. Taken together, it can be concluded that albuminuria in Cd-exposed people is a consequence of Cd-induced GFR fall, which causes SBP and DBP to rise along with the excretion rate of albumin.

4.2. Effects of Cadmium in Women and Men

By multiple regression analysis including all subjects, age, BMI, eGFR, Cd burden, diabetes, blood pressure, gender, and smoking contributed significantly to the variability of E_{alb}/C_{cr} among cohort participants (Table 4). In subgroup analysis, however, E_{alb}/C_{cr} inversely associated with eGFR in women only ($\beta = -0.188$ for SBP model and $\beta = -0.201$ for DBP model). An association of E_{alb}/C_{cr} with DBP was also found in women only ($\beta = 0.149$). The reasons for female preponderance effect of Cd on blood pressure and albuminuria were not apparent from the present study. A further research is warranted.

Typically, hypertension is more prevalent in men, compared to age-matched premenopausal women [52–54] and the differences of Cd effects in men and women were related to sex hormones. A 28% increase in serum testosterone levels in postmenopausal Japanese women was noted as their urinary Cd levels rose from <2 to ≥ 3 μg/g creatinine [55]. An inverse association between urinary Cd and serum estradiol levels was noted in postmenopausal Japanese and Swedish women [56,57]. In the Swiss Kidney Project on Genes in Hypertension [58], urinary Cd correlated with testosterone excretion in men, while there was a trend for an association in women.

4.3. Implications for Toxicological Risk Assessment of Dietry Cadmium Exposure

Hypertension, albuminuria, and CKD have been found repeatedly in people with a low body burden of Cd. A two-fold increase in risk of hypertension was associated with Cd excretion rate of 0.98 μg/g creatinine and a blood Cd level of 0.61 μg/L [59]. Similarly, an analysis of U.S. population data reported that the risk of having CKD increased 2.1-fold, 3.2-fold and 5.5-fold in people who had blood Cd concentrations of 0.21–0.35, 0.36–0.60, and > 0.60 μg/L, respectively [15].

Furthermore, given the same Cd body burden, the risk of having albuminuria was the highest (POR = 5.4) in diabetics with hypertension, compared to participants with severe tubular dysfunction plus hypertension (POR= 2.9) and participants with extremely severe tubular dysfunction plus hypertension (POR = 4.2) (Table 3). Thus, people with diabetes were highly susceptible to the nephrotoxicity of Cd. Consistent with our observation, a Dutch cross-sectional study, including 231 patients with type 2 diabetes, reported that low Cd exposure increased the risk of diabetic kidney disease [60]. In a six-year median follow-up of these patients, a progressive reduction of eGFR to kidney failure was linked to Cd exposure [61].

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In the present study, albuminuria was 3.4-time more prevalent than a low eGFR. This underscores the utility of an elevated albumin excretion of for early CKD detection purposes, given that CKD in its early stage is largely asymptomatic. This makes its early detection difficult and the initiation of early treatment, which can significantly prevent CKD progression, limited. ACR as low as 7 mg/g creatinine was a predictor of incident CKD within 10 years [36]. ACR ≥10 mg/g creatinine may increase mortality from all causes and CVD [37].

A significant increase in albumin excretion rate was found in participants with SBP and DBP within normal ranges (Figure 2). Participants with the lowest eGFR plus SBP ≥130 mmHg excreted albumin at 17.2% and 21.2% higher rates than those who moderate and high eGFR, respectively. Similarly, the lowest eGFR group with DBP ≥80 mmHg excreted albumin 19.0% and 22.5% higher rates than those in the moderate and high eGFR groups.

The extent of albumin excretion could thus serve as a sensitive toxicity endpoint for the toxicological risk assessment of Cd in the human diet. Current dietary CD exposure guidelines, based on a severe tubular dysfunction (Eβ2M/Ccr ≥ 3 μg/L filtrate or β_2M excretion rates ≥ 300 μg/g creatinine), are not protective of human health. There is an urgent need to develop new dietary Cd exposure limits.

5. Conclusions

Through the mediation analysis, this study shows, for the first time, that an elevation of albumin excretion rate is caused by an increase in blood pressure, notably SBP, which arises from Cd-induced GFR reductions. A declining eGFR, rising SBP and an elevated albumin excretion can serve as sensitive endpoints suitable for toxicological risk assessment of Cd in the human diet and the derivation of health-protective exposure limit.

Author Contributions: Conceptualization, S.S., D.A.V., and A.B.Đ.; methodology, S.Y., T.K., and P.P.; formal analysis, S.S. and A.B.Đ.; investigation, S.S., S.Y., T.K., and P.P.; resources, D.A.V., S.Y., and A.B.Đ.; data curation, S.S. and S.Y.; writing—original draft preparation, S.S.; writing—review and editing, S.S., D.A.V., and A.B.Đ.; project administration, S.S. and S.Y. All authors have read and agreed to the published version of the manuscript.

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