〈Original Article〉

Indoxyl sulfate is an independent risk factor for coronary heart disease in patients with chronic kidney disease

Shunji Namba[1], Yuko Okada[1], Masanari Sano[1], Tetsumi Kojima[1], Akiko Morimoto[1], Ippei Watanabe[2] and Toshisuke Morita[3]

Summary Background: Chronic kidney disease (CKD) has emerged as an independent risk for cardiovascular disease, possibly due to the effect of indoxyl sulfate (IS), a circulating uremic toxin. In the present study, we examined the relation between coronary heart disease (CHD) and serum IS concentration in patients with CKD.

Methods: The study comprised 39 patients with CHD (CHD group) and 41 without CHD (non-CHD group) who were treated for CKD in November 2010. The background and clinical characteristics of the groups were compared using receiver-operating characteristic analysis and multivariate analysis, and an IS cut-off value was calculated as an index of CHD risk.

Results: Serum IS concentration was significantly higher in the CHD group than in the non-CHD group ($P<0.0001$), and on multivariate analysis, IS was the strongest risk factor for CHD, as compared with factors such as smoking status, hypertension, diabetes mellitus, and estimated glomerular filtration rate. The adjusted odds ratio for CHD associated with elevated IS was 13.52. In patients with CKD, a serum IS concentration greater than 4.940 μ mol/L was the strongest risk factor for CHD.

Conclusion: CHD risk should be carefully considered in CKD patients with IS concentrations above this threshold.

Key words: Indoxyl sulfate, Uremic toxin, Chronic kidney diseases, Coronary heart disease, Risk factor

1. Introduction

Population-based cohort studies have identified chronic kidney disease (CKD) as an independent risk factor for cardiovascular disease (CVD) and coronary heart disease (CHD)[1, 2]. In CKD, the rates of CVD and CHD development increase due to cardiovascular abnormalities caused by progression of kidney disease. It is clear that cardio-renal interaction has an important pathophysiologic role in the development

[1]Department of Clinical Laboratory, Toho University Omori Medical Center
[2]Division of Cardiovascular Medicine, Department of Internal Medicine, Toho University
[3]Department of Laboratory Medicine, Toho University

Recieved for Publication April 10, 2014
Accepted for Publication April 16, 2014

Correspondence: Toshisuke Morita, Department of Laboratory Medicine, Toho University
6-11-1 Omori-Nishi, Ota-Ku, Tokyo 143-8541, Japan

of CVD and CHD; however, the mechanisms of this interaction are not well understood. Recent research has identified indoxyl sulfate (IS), a circulating uremic toxin, as a risk factor. IS is a metabolic end-product of tryptophan and is excreted into the urine from renal proximal tubules. Urinary excretion decreases with CKD progression, which leads to accumulation of IS in the body, the mean serum amount IS in the healthy subject was 2.390 μ mol/L and urinary excretion are about 2390.4 μ mol/L/day. Our previous study on IS and vascular cells clearly showed that IS causes inflammation, as well as oxidative stress on human umbilical-cord vein endothelial cells (HUVEC), thereby resulting in endothelial cell dysfunction[3]. Moreover, a correlation between serum IS concentration and arteriosclerosis was reported[4]. Thus, we examined whether IS is a risk factor for CHD development in patients with CKD and compared the risk of IS to those of established cardiovascular risk factors.

2. Methods

1. Patient selection

We selected a random sample from the 636 patients with CKD who consulted our department of cardiovascular internal medicine in November 2010. We reviewed clinical characteristics and findings from electrocardiography, chest radiography, cardiac ultrasound, nuclear medicine studies, and blood testing. A diagnosis of CHD was confirmed in 351 patients. Patients with complete information on total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (Glu), hemoglobin A1c [HbA1c (NGSP)] concentration, and estimated glomerular filtration rate (eGFR) were included: 39 had CHD (12 cases of acute myocardial infarction, 13 cases of old myocardial infarction, 14 cases of angina), and 41 had no history of CHD.

We excluded patients receiving dialysis, those with missing data on patient characteristics current (smoking habit, age, hypertension, diabetes mellitus, medical history of hyperlipemia), and those receiving treatment with an oral adsorbent. This study adhered to the 2001 guidelines for protection of personal information in clinical studies, published by the Japanese Society of Laboratory Medicine.

2. Laboratory testing

In blood testing, we collected data on serum TC, HDL-C, LDL-C, TG, Glu and HbA1c concentration. Serum IS concentration was assayed with a high-

Table 1 Clinical and demographic characteristics of the study population

Characteristics	All (n=80)	non-CHD (n=41)	CHD (n=39)	P-value
Age (yr)	68.5±10.7	66.7±11.6	70.3±9.5	0.1253
Male gender [n(%)]	50 (62.5)	23 (56.1)	27 (69.2)	0.1867
Smoking habit [n(%)]	33 (41.3)	14 (34.1)	19 (48.7)	0.1903
HTN [n(%)]	26 (32.5)	13 (31.7)	13 (33.3)	0.8786
SBP (mmHg)	129.2±23.1	132.6±24.2	125.9±21.8	0.2583
DBP (mmHg)	75.5±15.2	79.5±16.5	71.7±12.9	0.0443
DM [n(%)]	17 (21.3)	7 (17.1)	10 (25.6)	0.2387
CKD stage [n(%)]				
1	13 (16.3)	8 (19.5)	5 (12.8)	0.3740
2	26 (32.5)	14 (34.1)	12 (30.8)	0.4256
3	41 (51.3)	19 (46.3)	22 (56.4)	0.2612

Abbreviation: non-CHD, non-coronary heart disease; CHD, coronary heart disease; HTN, hypertension; SBP, systolic blood pressure; DBP, Diastolic blood pressure; DM, diabetes mellitus; CKD, chronic kidney disease.

Data are expressed as mean±SD or, for binary variables, number (frequency).

P value versus non-CHD, CHD.

performance liquid chromatography method[5]. The reference serum IS concentration, as defined by measurements in healthy subjects (n= 40), was defined as 2.390±3.586 μ mol/L.

The eGFR (mL/min/1.73 m^2) was calculated using the following equation:

eGFR=194 × serum $Cr^{-1.094}$ × $age^{-0.287}$ × (0.739, if female)

3. Statistical analysis

Data are expressed as mean ± standard deviation. The chi-square test was used to analyze differences between groups, and continuous variables were analyzed with the Student t-test. A P-value of 0.05 or less was considered to indicate statistical significance in all tests. The risk of CHD was assessed by multivariate logistic regression. In the present analysis, putative risk factors with a P value of 0.05 or less on univariate analysis and a relatively high odds ratio (OR) were included in multivariate analysis. Statview Version 5.0 statistical software (SAS Institute Inc. Cary, NC, USA) was used for all analyses.

3. Results

The characteristics of patients with and without CHD are shown in Table 1. Mean age was 68.5.4± 10.7 years, 50 were male, and 30 were female, 33 were current smokers, 26 had hypertension, and 17 had diabetes mellitus. With regard to CKD stage, 13 had stage 1, 26 had stage 2, and 41 had stage 3 CKD. There was no significant difference with regard to CHD status in age, male-to-female ratio, smoking status, hypertension, SBP, diabetes or CKD stage. However, the proportions of patients DBP than 7.8 mmHg (P=0.0443) were lower in the non-CHD group than in the CHD group.

IS concentration in the non-CHD group was 3.705±2.669 μ mol/L as compared with 8.805± 5.219 μ mol/L in the CHD group (P<0.0001). Moreover, glucose was higher, and TC, LDL-C, and eGFR were lower, in the CHD group (Table 2). In the non-CHD group, IS concentration ranged from 0.120 to 14.542 μ mol/L, the concentration in first quartile (Q1) was 1.952 μ mol/L, the median was 3.347 μ mol/L, and the concentration in third quartile (Q3) was 4.781 μ mol/L. In the CHD group, IS concentration ranged from 2.231 to 24.462 μ mol/L, Q1 concentration was 4.462 μ mol/L, the median was 8.287 μ mol/L, and the Q3 concentration was 11.355 μ mol/L. All these values were significantly higher than the corresponding values in the non-CHD group (P<0.0001) (Fig. 1).

IS concentration and eGFR were strongly negatively correlated (r=−0.468; P＜0.0001). Analysis of causal relationship between CHD and IS: On multivariate analysis the unadjusted OR for CHD were 14.85 (4.88-45.15; P<0.0001) for IS. The

Table 2 Biochemical characteristics of the study population

Biochemical markers	All (n=80)	non-CHD (n=41)	CHD (n=39)	P-value
T-CHO (mmol/L)	4.74±0.97	5.03±0.96	4.45±0.90	0.0130
HDL-C (mmol/L)	1.56±0.45	1.62±0.50	1.52±0.40	0.4123
LDL-C (mmol/L)	2.80±0.88	3.15±0.84	2.53±0.83	0.0091
TG (mmol/L)	1.29±0.85	1.29±0.88	1.29±0.83	0.9855
Glu (mmol/L)	74.7±38.7	66.1±20.1	82.8±48.7	0.0670
HbA1c (%)	6.00±0.84	5.74±0.71	6.16±0.911	0.0763
eGFR (mL/min/1.73m^2)	64.1±19.9	67.5±22.2	60.4±16.6	0.1118
IS (μ mol/L)	6.175±4.821	3.705±2.669	8.725±5.259	＜0.0001

Abbreviation: non-CHD, non-coronary heart disease; CHD, coronary heart disease; T-CHO, total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TG, triglycerides; Glu, Glucose; HbA1c, glycosylated hemoglobin A1c; eGFR, estimated glomerular filtration rate; IS, indoxyl sulfate.
Data are expressed as mean±SD. P value versus non-CVD,CVD.

OR for IS was the largest of any factor. In addition, although not statistically significant, 11 other factors had ORs that were larger than unity: age, smoking, hypertension, diabetes, T-CHO, TG, HDL-C, LDL-C, Glu, HbA1c, and eGFR (Table 3). These 6 items were then added to the multivariate model to confirm that IS was an independent risk factor. However, the adjusted OR was almost unchanged, at 13.52 (3.05-59.94; P=0.0006) (Table 4).

The cut-off value of IS was set to 4.940 μ mol/L, which yielded a sensitivity of 74.4 %, and a specificity of 82.9 %. The area under curve (AUC), an index of test performance in distinguishing CHD, was 0.825 (Fig. 2).

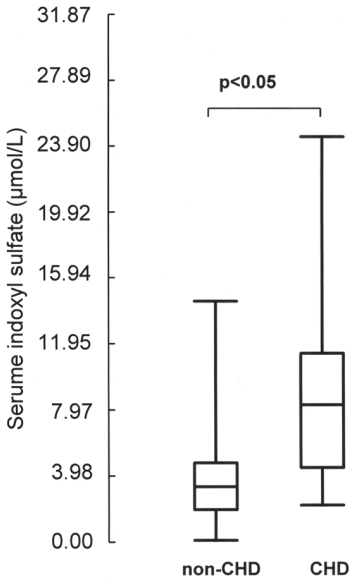

Fig. 1 Comprision serum levels of IS between non-CHD and CHD.
Abbreviation: non-CHD, non-coronary heart disease; CHD, coronary heart disease
Data are expressed as minimum, first quartile, median, third quartile and maximum.
The dotted line indicates the reference value for healthy controls.

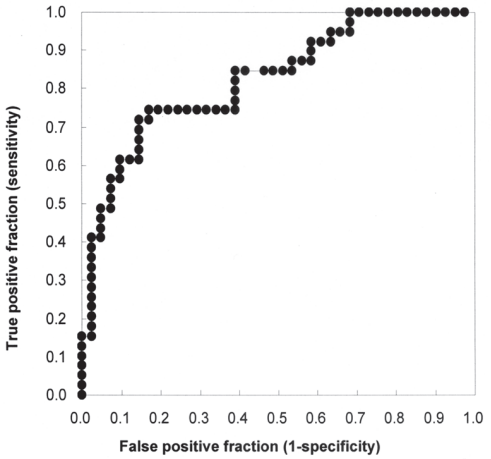

Fig. 2 Receiver operating characteristic curve analysis for serum indoxyl sulfate (area under the curve = 0.825) to detect coronary heart disease.

Table 3 Univariate analysis of variables associated with CHD in CKD patients

Variables	Regression coefficient	Standard error	Odds ratio	95%Confidence limit	P- value
Age	0.034	0.022	1.03	0.99-1.08	0.1275
Smoking habit	0.605	0.459	1.83	0.74-4.51	0.1876
HTN	0.074	0.478	1.01	0.42-2.75	0.8767
DM	0.646	0.547	1.91	0.65-5.57	0.2373
T-CHO	-2.326	1.094	0.10	0.01-0.83	0.0334
TG	-0.258	0.543	0.77	0.27-2.24	0.6346
HDL-C	-0.210	0.561	0.81	0.27-2.44	0.7082
LDL-C	-0.560	0.643	0.57	0.16-2.02	0.3843
Glu	0.580	0.480	1.79	0.70-4.58	0.2270
HbA1c	0.916	0.581	2.50	0.80-7.81	0.1147
eGFR	-0.019	0.012	0.98	0.96-1.01	0.1141
IS	2.698	0.567	14.85	4.88-45.15	＜0.0001

Abbreviation: CHD, coronary heart disease; CKD, chronic kidney disease; HTN, hypertension; DM, diabetes mellitus; T-CHO, total cholesterol; TG, triglycerides; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; Glu, glucose; HbA1c, glycosylated hemoglobin A1c; eGFR, estimated glomerular filtration rate; IS, indoxyl sulfate.
Odds ratio are expressed as non-adjusted odds ratio.

4. Discussion

It is believed that, in addition to conventional cardiovascular risk factors, there is a factor peculiar to CKD that increases the risk of CHD. Although attempts have been made to explain the mechanism responsible for the relation between CHD and CKD there are many factors involved in humoral regulation and endothelial function. For example, progression of arteriosclerosis accompanied by vascular calcification is closely related to cardiac hypertrophy due to abnormalities in humoral regulation, or heart failure and calcium-phosphate metabolism etc. However, such changes can also cause subclinical endothelial dysfunction in CKD[6] which could explain why the prevalence of arteriosclerosis is high among individuals with CKD.

Masai et al. reported in their study of endothelial cells that IS causes oxidative stress via NADPH oxidase, activates nuclear factor- κ B, and regulates production of monocyte chemoattractant protein-1 in HUVEC[3]. In addition, Barreto et al. found a positive correlation between IS concentration in patients with CKD and indices of arteriosclerosis, eg, pulse wave velocity[4]. These results appear to confirm those from the above in vitro study. Moreover, IS damages glomerular mesangial cells and renal tubule cells and promotes glomerulosclerosis and formation of obstructions between nephric tubules[7-11]. These pathologic changes are believed to be due to IS-induced production of reactive oxygen species, which cause oxidative stress in various cells and tissues[3, 12]. Thus, although there have been studies of the mechanism by which IS stimulates inflammation and increases oxidative stress to cells in the cardiovascular system, thereby causing endothelial cell dysfunction and consequent arteriosclerosis, the relation between CHD development and CKD has not been adequately discussed. Our findings indicate that IS concentration is an important risk factor for CHD and that it should be closely monitored in patients with CKD.

In our comparison of CKD patients with and without CHD, serum IS concentration was significantly higher in patients with CHD, and the risk of CHD associated with IS was high in univariate and multivariate analyses, which suggests that IS is the most important risk factor for CHD in this patient population. The IS concentration was 2.390 μ mol/L in healthy adults, 3.466 μ mol/L in the non-CHD group, and 8.725 μ mol/L in the CHD group. With CHD as the dependent variable and smoking, blood test values, and background factors, such as hypertension, as the independent variables, the unadjusted OR on univariate analysis was 14.85 (P<0.0001), which was much higher than the ORs of the other factors, which ranged from 0.10 to 2.50. Furthermore, the adjusted OR for IS remained high, 13.52 (P= 0.0006), even after adjustment for smoking status, hypertension, diabetes mellitus, glucose level, and HbA1c in multivariate models. Thus, we conclude that IS is the most important risk factor for CHD development. For this

Table 4 Multivariate analysis of variables associated with CHD in CKD patients

Variables	Regression coefficient	Standard error	Odds ratio	95% Confidence limit	P-value
Smoking habit	1.462	0.682	4.32	1.13-16.42	0.0259
HTN	-0.302	0.684	0.74	0.19-2.83	0.6580
DM	-0.168	0.745	0.85	0.20-3.64	0.8217
Glu	-0.390	0.700	0.68	0.17-2.67	0.5744
HbA1c	1.207	0.762	3.34	0.75-14.87	0.1057
eGFR	-0.012	0.017	0.99	0.96-1.02	0.4672
IS	2.604	0.760	13.52	3.05-59.95	0.0006

Abbreviation: CHD, coronary heart disease; CKD, chronic kidney disease; HTN, hypertension; DM, diabetes mellitus; Glu, glucose; HbA1c, glycosylated hemoglobin A1c; eGFR, estimated glomerular filtration rate ; IS, indoxyl sulfate.

Odds ratio are expressed as adjusted odds ratio.

reason, we established an IS level of 4.940 μ mol/L as a cut-off value in the assessment of CHD development. Therefore, it is necessary to carefully monitor CKD patients with an IS concentration greater than 4.940 μ mol/L.

In conclusion, as compared with traditional cardiovascular risk factors, IS was a greater risk factor for CHD development among patients with CKD. Thus, CKD patients with an IS concentration greater than 4.940 μ mol/L should be carefully monitored for development of CHD.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

1. Parikh NI, Hwang SJ, Larson MG, Levy D, Fox CS: Chronic kidney disease as a predictor of cardiovascular disease (from the Framingham Heart Study). Am J Cardiol, 102: 47-53, 2008.
2. Ninomiya T, Kiyohara Y: Chronic kidney diseases looked from the Hisayama Studies. Research Clinical, 55: 1248-1254, 2006.
3. Masai N, Tatebe J, Yoshino G, Morita T: Indoxyl sulfate stimulates monocyte chemoattractant protein-1 expression in human umbilical vein endothelial cells by inducing oxidative stress through activation of the NADPH oxidase-nuclear factor-kappa B pathway. Circ J, 74: 2216-2224, 2010.
4. Barreto FC, Barreto DV, Lisbeuf S, Meert N, Glorieux G, Temmar M, Choukroun G , Vanholder R, Massy ZA: Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clin J Am Soc Nephrol, 4: 1551-1558, 2009.
5. Niwa T, Miyazaki T, Tsukushi S, Maeda K, Tsubakihara Y, Owada A, Shiigai T: Accumulation of indoxyl- β -D-glucuronide in uremic serum. Nephron, 74: 72-78, 1996.
6. Kai H, Kajimoto H, Imaizumi T: Blood vessel renal and asymmetric dimethylarginine. J Jpn Coll Angiol, 50: 659-664, 2010.
7. Gelasco AK, Raymond JR: Indoxyl sulfate induces complex redox alterations in mesangial cells. Am J Physiol, 290: 1551-1558, 2006.
8. Motojima M, Hosokawa A, Yamato H, Muraki T, Yoshioka T: Uremic toxins induce proximal tubular injury via organic anion transporter 1-mediated uptake. Br J Pharmacology, 135: 555-563, 2002.
9. Niwa T, Ise M: Indoxyl sulfate, a circulating uremic toxin, and stimulates the progression of glomerular sclerosis. J Lab Clin Med, 24: 96-104, 1994.
10. Miyazaki T, Ise M, Endo K, Ito Y, Seo H, Niwa T: Indoxyl sulfate stimulates renal synthesis of transforming growth factor-beta 1 and progression of renal failure. Kidney Int Suppl, 63: 211-214, 1997.
11. Niwa T, Aoyama I, Takayama F, Tsukushi S: Urinary indoxyl sulfate is a clinical factor that affects the progression of renal failure. Mine Electrolyte Metab, 25: 118-122, 1999.
12. Dou L, Jourd CN, Faure V, Cerini C, Berland Y, Dignat GF, Brunet P: The uremic solute indoxyle sulfate induces oxidative stress in endothelial cells. J Thromb Haemost, 5: 1302-1308, 2007.