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Association Between Indoxyl Sulfate and Cardiac Dysfunction and Prognosis in Patients With Dilated Cardiomyopathy

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Background: Serum indoxyl sulfate (IS) is a uremic toxin that accelerates the progression of chronic kidney disease (CKD). The aim of this study was to determine whether serum IS is associated with hemodynamic parameters or cardiac events in patients with nonischemic dilated cardiomyopathy (DCM).

Methods and Results: The 76 patients with DCM had their serum IS and plasma brain natriuretic peptide (BNP) levels measured, and underwent echocardiographic examination. Mean (±standard deviation) left ventricular ejection fraction (LVEF) and BNP levels in the patients were 32.5±10.7% and 204±219 pg/ml, respectively. Patients were divided into 2 groups, low IS (<0.9 μg/ml) and high IS (≥0.9 μg/ml), based on the median value of serum IS. Although there were no significant differences in LVEF and BNP between the groups, E/e' was significantly greater in the high IS group than in the low IS group. Furthermore, E/e' was an independent determinant of serum IS level. The risk of a cardiac event was significantly higher in the high IS group than in the low IS group (P=0.014). Moreover, serum IS was a significant predictor of cardiac events even after adjustment for BNP.

Conclusions: Cardiac dysfunction is associated with the serum IS level, which might serve as a new prognostic marker in DCM patients with normal renal function or mild to moderate CKD.

Key Words: Cardiovascular disease; Chronic kidney disease; Heart failure; Mortality; Uremia

Nonischemic dilated cardiomyopathy (DCM) is a heart muscle disease in which one or both ventricles become enlarged, and the pumping function is decreased.[1] Despite progress in pharmacotherapy for heart failure (HF), the prognosis of individuals with DCM is still poor, so it is important that DCM patients who are refractory to standard medical treatment be placed under strict management as early as possible. Several studies have attempted to identify abnormalities associated with poor prognosis in individuals with DCM.[2–7] More efficient, less expensive, and simpler methods are required for diagnosis and risk assessment in these patients. Plasma brain natriuretic peptide (BNP) is 1 of the most powerful predictors of cardiac events in patients with DCM,[6,8] but few other biomarkers that are suitable as prognostic markers for HF have been identified.

Indoxyl sulfate (IS) is a uremic toxin that accelerates the progression of chronic kidney disease (CKD).[9] Tryptophan derived from dietary protein is metabolized to indole by tryptophanase, which is produced by intestinal bacteria such as *Escherichia coli*. Indole is absorbed into the blood from the intestine and metabolized to IS in the liver, normally being excreted into urine; however, in uremia, reduced renal clearance of IS leads to elevated serum levels, such as is observed in patients with CKD.[10,11] Barreto et al suggested that IS might play a significant role in the vascular disease and high mortality observed in CKD patients.[12] However, the association of IS and cardiac dysfunction remains uncertain, as does its use as a prognostic marker for HF patients.

The prevalence of renal dysfunction in individuals with chronic HF is approximately 25%[13] and CKD contributes to reduced cardiac function, cardiac hypertrophy, and an increased risk of adverse cardiovascular events. We hypothesized that high serum IS levels would be associated with cardiac dysfunction and poor prognosis. To the best of our knowledge, there are no reports on the relationship between serum IS levels and prognosis in patients with DCM, so the aim of the present study was to determine whether the serum IS level is associated with hemodynamic parameters, and whether it is a predic-

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Table 1. Baseline Clinical and Hemodynamic Characteristics, and Cardiac Events During Follow-up

	All (n=76)	Low IS (n=38)	High IS (n=38)	P value
Age (years)	53.1±13.7	50.5±13.1	55.7±14	0.097
Sex (male, %)	71	68	74	0.613
BMI (kg/m^2)	23.7±4.3	23.7±4.3	23.7±4.3	0.994
SBP (mmHg)	121.0±23.5	117.7±23.6	124.3±23.3	0.087
NYHA class I/II/III/IV (n)	39/31/5/1	21/14/2/1	18/17/3/0	0.434
Hyperlipidemia (n, %)	23 (30)	10 (26)	13 (34)	0.309
Diabetes mellitus (n, %)	20 (26)	8 (21)	12 (32)	0.297
Smoking (n, %)	18 (24)	11 (29)	7 (18)	0.280
Medication				
Diuretics (n, %)	45 (59)	16 (42)	29 (76)	0.010
β-blockers (n, %)	47 (62)	19 (50)	28 (74)	0.102
ACE inhibitors (n, %)	17 (22)	6 (16)	11 (29)	0.169
ARBs (n, %)	31 (41)	13 (34)	18 (47)	0.243
Statins (n,%)	9 (12)	2 (5)	7 (18)	0.076
Digitalis (n, %)	9 (12)	5 (13)	4 (11)	0.723
Aldosterone antagonists (n, %)	30 (39)	9 (24)	21 (55)	0.050
Amiodarones (n, %)	6 (8)	2 (5)	4 (11)	0.644
Laboratory measurements				
Hemoglobin (mg/dl)	14.1±1.7	14.3±1.5	13.9±1.8	0.312
Creatinine (mg/dl)	0.90±0.25	0.81±0.24	0.98±0.23	0.003
Estimated GFR (ml·min^{-1}·1.73 m^{-2})	71.4±24.5	79.3±24.6	63.4±21.9	0.004
Plasma BNP (pg/ml)	208±219	220±248	195±189	0.618
Electrocardiography				
Heart rate (beats/min)	75±15	76±14	74±14	0.667
QRS duration (ms)	112.9±22.7	107.5±15.2	118.5±27.4	0.039
Echocardiography				
LVDd (mm)	61±9.3	61±9.3	61.3±9.4	0.903
LVDs (mm)	51±11.1	51±11.1	51.2±11.1	0.954
IVS (mm)	9.4±2.3	9.3±2.3	9.4±2.2	0.897
LVPW (mm)	9.2±2.3	8.8±1.8	9.5±1.9	0.124
LVEF (%)	32.5±10.7	32.3±11.8	32.6±9.7	0.909
LV mass index (g/m^2)	164±56	158±49	169±62	0.388
LAD (mm)	40.9±8.1	41.1±8.1	40.7±8.3	0.841
DCT (ms)	188.0±54.8	182.3±44.5	193.5±63.5	0.416
E/A	1.36±1.04	1.38±0.98	1.33±1.09	0.847
E/e'	14.4±6.5	12.3±4.7	16.4±7.3	0.008
Cardiac events during follow-up				
Biventricular pacing (n, %)	3 (4)	0 (0)	3 (8)	0.120
ICD (n, %)	2 (3)	0 (0)	2 (5)	0.247
Heart failure (n, %)	15 (20)	3 (8)	12 (32)	0.009
Death (n, %)	5 (7)	2 (5)	3 (8)	0.500

Data are presented as means ± SD.
ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; DCT, deceleration time; E/e', ratio of early transmitral velocity to tissue Doppler mitral annular velocity during early diastole; GFR, glomerular filtration rate; ICD, implantable cardioverter defibrillator; IS, indoxyl sulfate; IVS, intraventricular septal thickness diameter; LAD, left atrial diameter; LV, left ventricle; LVDd, LV diastolic diameter; LVDs, LV systolic diameter; LVEF, LV ejection fraction; LVPW, LV posterior wall thickness; NYHA, New York Heart Association; SBP, systolic blood pressure.

tor of cardiac events in patients with DCM. We focused only on patients with DCM because, if patients with cardiac dysfunction caused by ischemic cardiomyopathy were included, the ischemic heart disease could affect prognosis and conceal the effect of IS.

Methods

Study Population

We enrolled 76 patients with DCM (54 men, 22 women) in the study. DCM was defined by the presence of left ventricular ejection fraction (LVEF) <50% (as determined by echocardiography), and the absence of coronary artery stenosis (as determined by coronary angiography or computed tomography coronary angiography), valvular heart disease, arterial hyper-

Table 2. Univariate and Multivariate Linear Regression Analyses for Indoxyl Sulfate

	Univariate		Multivariate	
	r	P value	β (95% CI)	P value
Age (years)	0.315	0.006	0.004 (–0.007 to 0.015)	0.438
Sex (male: 0, female: 1)	0.123	0.279		
BMI (kg/m^2)	–0.048	0.681		
SBP (mmHg)	0.147	0.231		
Heart rate (beats/min)	–0.059	0.634		
Hemoglobin (mg/dl)	–0.182	0.115		
Creatinine (mg/dl)	0.298	0.009		
Estimated GFR (ml·min^{-1}·1.73 m^{-2})	–0.382	0.001	–0.007 (–0.013 to –0.002)	0.013
Plasma BNP (pg/ml)	0.006	0.958		
QRS duration (ms)	0.317	0.005	0.003 (–0.003 to 0.009)	0.345
LVDd (mm)	–0.020	0.863		
LVDs (mm)	0.006	0.962		
LV mass index	0.036	0.756		
LVEF (%)	–0.001	0.995		
LAD (mm)	–0.072	0.539		
E/e'	0.423	<0.001	0.260 (0.003 to 0.050)	0.027

CI, confidence interval. Other abbreviations as in Table 1.

tension, and secondary cardiac muscle disease attributable to any known systemic conditions.[14] All the patients underwent laboratory measurements and echocardiography. The patients were followed up prospectively for cardiac death (death from worsening of HF or sudden death) for a mean of 32 months (range, 1.5–61 months). Cardiac events were defined as cardiac death or hospitalization for worsening of HF. The Ethical Review Board of Nagoya University School of Medicine approved the study protocol and all subjects provided written informed consent with regard to the study procedures and potential risks.

The estimated glomerular filtration rate (eGFR) at baseline was calculated using the revised equation for Japanese individuals,[15] which incorporates age, sex, and serum creatinine concentration: eGFR (ml·min^{-1}·1.73 m^{-2}) for males was calculated as $194\times(\text{serum creatinine concentration})^{-1.094}\times(\text{age})^{-0.287}$, whereas that for females was calculated with the same equation, with the result multiplied by a correction factor of 0.739. Patients with eGFR <30 ml·min^{-1}·1.73 m^{-2} were excluded from the study.

Serum IS levels were measured by using high-performance liquid chromatography (HPLC).[11] Each serum sample was filtered through a 0.20-μm membrane, and 10-μl samples of the filtrates were analyzed by reversed-phase HPLC (Shiseido Capcell Pak MF Ph-1 SG80 MF 150×4.6 mm; Shiseido, Tokyo, Japan). The mobile phase, 0.1 mol/L KH_2PO_4/tetrahydrofuran (95/5), was delivered at a flow rate of 1.0 ml/min at 35°C. The serum IS levels were determined by fluorescence detection (excitation, 295 nm; emission, 390 nm).

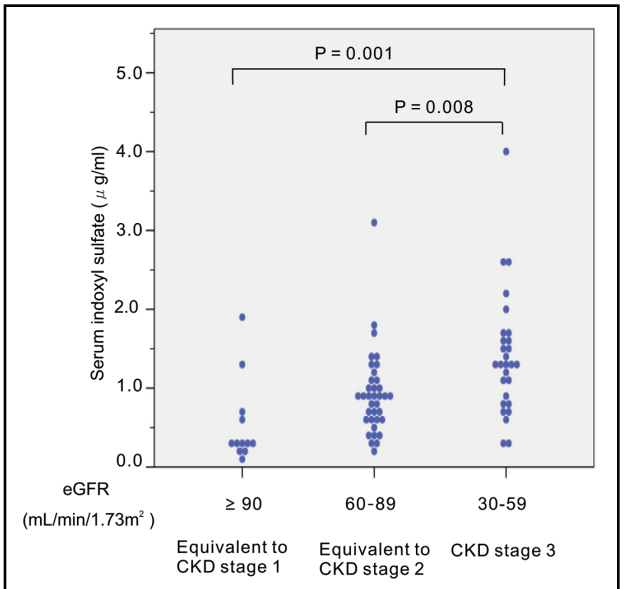

Figure 1. Serum indoxyl sulfate levels in patients with different stages of kidney function classified by estimated glomerular filtration rate (eGFR; equivalent to CKD stage 1: eGFR ≥90 ml·min^{-1}·1.73 m^{-2}, equivalent to CKD stage 2: 60–89 ml·min^{-1}·1.73 m^{-2}, and CKD stage 3: 30–59 ml·min^{-1}·1.73 m^{-2}). CKD, chronic kidney disease.

Echocardiography

Standard M-mode and 2-dimensional echocardiography, Doppler blood flow, and tissue Doppler imaging measurements were performed in agreement with the American Society of Echocardiography guidelines, using the Vivid 7 system (GE Healthcare, Milwaukee, WI, USA).[16] Septal and posterior LV wall thicknesses were obtained from the parasternal long-axis view. The LV end-diastolic and end-systolic volumes were obtained from 4 apical and 2 chamber views.[17] The LVEF was calculated from 2-dimensional apical images according to the modified Simpson's method. Pulse-wave Doppler echocardiography was used to assess mitral peak early (E) and late wave flow velocity and E-wave deceleration time. The tissue Doppler imaging wave sample of the mitral annulus was obtained from the septal side of the apical 4-chamber view. The early (e') diastolic peak velocity was calculated. The ratio of early transmitral flow velocity to early diastolic mitral annular velocity (E/e') was taken as an estimate of LV filling pressure.[18]

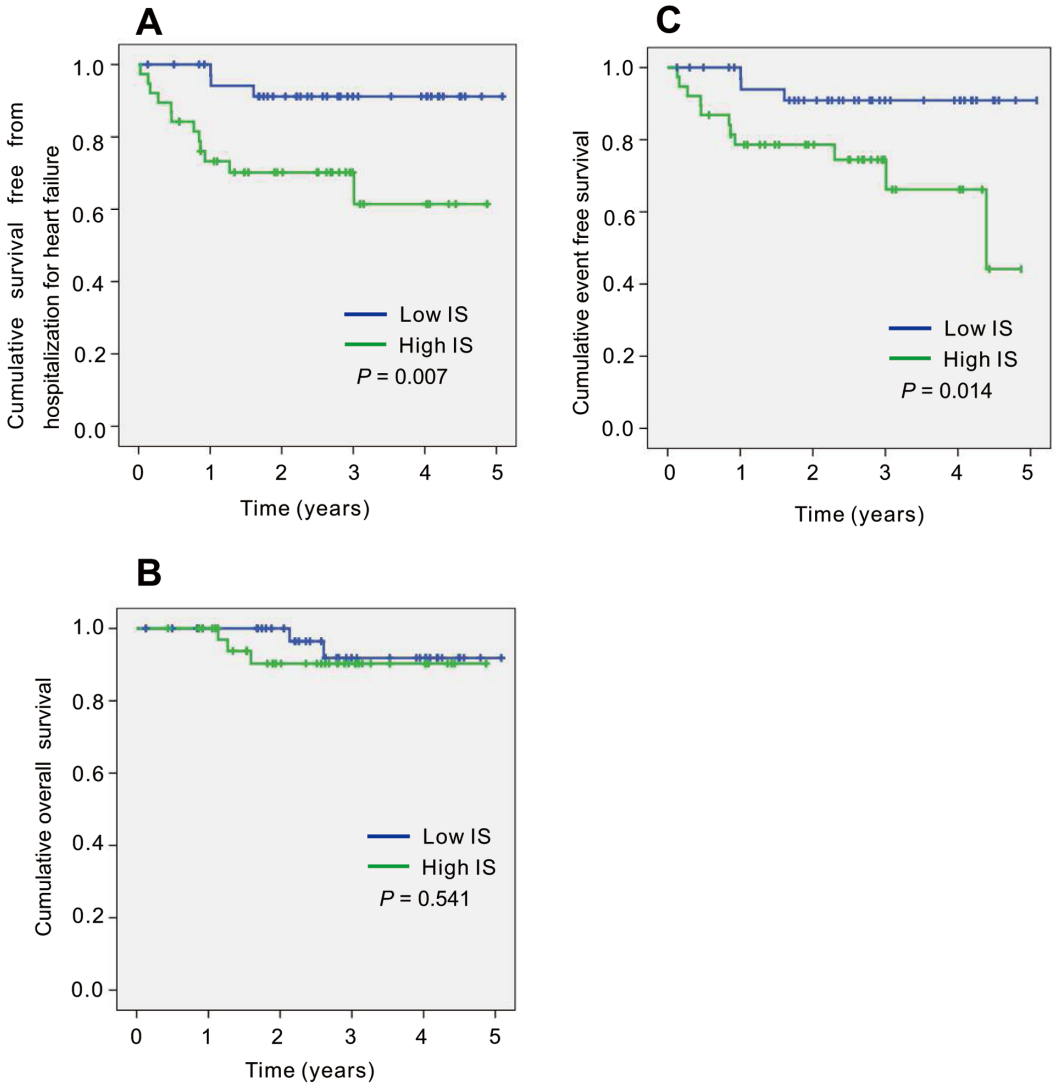

Figure 2. Kaplan-Meier analyses of (**A**) cumulative survival free from hospitalization for heart failure, (**B**) cardiac death-free cumulative survival (ie, overall cumulative survival, because no patients died from other causes during the follow-up), and (**C**) cardiac event-free cumulative survival (cardiac events were defined as cardiac death or hospitalization for worsening heart failure), in the low and high indoxyl sulfate (IS) groups.

Statistical Analysis

Data are presented as mean±SD. Continuous variables were compared between the low and high IS groups with the use of Student's t-test for unpaired data. Categorical variables are presented as numbers (percentages), and comparisons between the 2 groups were performed by the chi-squared test (or Fisher's test where appropriate). Pearson's correlation coefficient was used to assess relationships between serum IS levels and selected clinical or biochemical variables. We used univariate and multivariate linear regression analyses to identify factors that were independently associated with serum IS; the multivariate model included all baseline valuables that had a significant correlation with IS in the univariate analysis. Cumulative cardiac event-free survival estimates were calculated by using the Kaplan-Meier method. Differences between survival curves were assessed by the log-rank test. Cox proportional hazard regression analysis was performed to calculate the hazard ratio (HR) and 95% confidence interval for cardiac events. In Cox proportional hazards analysis, HRs were calculated after just adjusting for plasma levels of BNP. Multivariate Cox proportional hazard regression analyses were performed to adjust for various factors, such as age, sex, eGFR, hemoglobin, plasma BNP level, QRS duration and E/e'. All analyses were performed with the SPSS 19.0 software package (SPSS/IBM, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

Comparison Between Low and High IS Groups

Patients were divided into 2 groups on the basis of their median serum IS values (low IS group, <0.9 μg/ml; high IS group,

Table 3. Multivariate Cox Regression Analysis for Cardiac Events Adjusted for BNP

	HR	95% CI	P value
BNP (crude (pg/ml))	1.003	1.001–1.004	0.001
Age (years)	0.978	0.938–1.019	0.282
Sex (male: 0, female: 1)	0.930	0.863–1.002	0.065
SBP (mmHg)	1.005	0.983–1.027	0.652
Heart rate (beats/min)	1.009	0.978–1.048	0.660
Hemoglobin (mg/dl)	0.774	0.554–1.082	0.134
Creatinine (mg/dl)	1.227	0.103–15.81	0.849
eGFR (ml·min^{-1}·1.73 m^{-2})	1.012	0.988–1.036	0.329
IS (continuous variable)	1.746	1.017–2.999	0.043
QRS duration (ms)	1.020	1.003–1.038	0.023
LVDd (mm)	0.981	0.921–1.045	0.555
LVEF (mm)	1.001	0.950–1.055	0.976
DCT (ms)	0.991	0.975–1.007	0.250
e' (cm/s)	0.882	0.650–1.198	0.421
E/A	0.788	0.398–1.561	0.494
E/e'	1.004	0.927–1.089	0.917

HR, hazard ratio. Other abbreviations as in Tables 1,2.

Table 4. Multivariate Cox Regression Analysis for Cardiac Events, With IS Entered as Categorical Data (Low or High)

	HR	95% CI	P value
Univariate	4.29	1.19–15.44	0.026
Multivariate			
Model 1	5.68	1.54–20.94	0.009
Model 2	19.94	3.22–123.60	0.001
Model 3	17.74	2.12–148.22	0.008

Model 1 is adjusted for age and sex.
Model 2 is adjusted for age, sex, hemoglobin, eGFR, and BNP.
Model 3 is adjusted for age, sex, hemoglobin, eGFR, BNP, QRS duration, and E/e'.
Abbreviations as in Tables 1–3.

≥0.9 μg/ml). The mean serum IS level in the study population was 1.04±0.70 (range, 0.08–4.0 μg/ml). Baseline clinical characteristics of all the patients are listed in **Table 1**. There were no significant differences between the 2 groups in age, sex, or body mass index. With the exception of diuretics, there were no significant differences in drug treatment at entry into the study. The high IS group showed significantly higher serum creatinine levels and lower eGFR values than the low IS group. There were no significant differences between the 2 groups in plasma BNP levels. QRS duration was significantly longer in the high IS group than in the low IS group (118.5±27.4 ms vs. 107.5±15.2 ms, P=0.039). E/e' was significantly higher in the high IS group than in the low IS group (16.4±7.3 vs. 12.3±4.7, P=0.008). With the exception of E/e', there were no significant differences in the echocardiography data of the 2 groups.

Correlation Between IS and Clinical Parameters

The results of univariate and multivariate linear regression analyses of the relationship between various clinical variables and IS are listed in **Table 2**. In the univariate analysis, a significant positive correlation was observed for age, serum creatinine, QRS duration, and E/e', and a significant negative correlation was observed for eGFR. Neither LVEF nor plasma BNP correlated with serum IS. These 5 variables were then included in the multivariate analysis of IS, and only E/e' and eGFR were found to be significant independent determinants of IS.

Figure 1 shows serum IS levels in patients with different stages of kidney function classified by eGFR (equivalent to stage 1 CKD: eGFR ≥90 ml·min^{-1}·1.73 m^{-2}, equivalent to stage 2 CKD: 60–89 ml·min^{-1}·1.73 m^{-2}, and stage 3 CKD: 30–59 ml·min^{-1}·1.73 m^{-2}). There were no patients with CKD stage 4 or 5 in this study. Serum IS significantly increased as CKD progressed.

The 2 clinical outcomes (hospitalization for worsening of HF and cardiac death) were assessed by the Kaplan-Meier method and log-rank test (**Figure 2**). The probability of hospitalization for HF was higher in the high IS group than in the low IS group (P=0.007) (**Figure 2A**). In contrast, there was no significant difference between the 2 groups for the probability of cardiac death (**Figure 2B**). The probability of a cardiac event (hospitalization for HF, cardiac death, or both) was higher in the high IS group than in the low IS group (P=0.014; **Figure 2C**).

Cox proportional hazard regression analyses of survival free from cardiac events after only adjusting for BNP are shown in **Table 3**. Plasma BNP (HR 1.003, P=0.001), IS (HR 1.746, P=0.043), and QRS duration (HR 1.020, P=0.023) were identified as independent contributing factors for cardiac events. We performed univariate and multivariate Cox analyses with IS as a categorical variable (**Table 4**). After adjustment for age, sex, hemoglobin, eGFR, BNP, QRS duration, and E/e' (model 3), IS remained an independent predictor for cardiac events (HR 17.74; P=0.008).

Discussion

In the present study, we demonstrated that an elevated serum IS level was associated with both E/e' and hospitalization for worsening of HF in DCM patients with normal renal function or mild to moderate CKD. These effects were independent of age, sex, hemoglobin, eGFR, BNP, QRS duration, and E/e'.

Serum IS as a Prognostic Marker

The serum IS level is associated with oxidative stress in CKD and vascular diseases,[19,20] and is associated with cardiovascular mortality in CKD patients.[12] Therefore, we investigated whether serum IS could be a useful prognostic marker in DCM patients. By conducting multivariate Cox proportional hazard regression analyses with several important clinical parameters, including plasma BNP levels, as explanatory variables, we demonstrated that serum IS was an independent predictor of cardiac events. This finding suggests that serum IS could be used in addition to BNP as a useful predictor of prognosis in ambulatory patients with DCM and normal kidneys or mild to moderate kidney disease.

The average serum IS level in our study was lower than that in the previous studies.[12,21] We previously reported serum IS levels of 0.64±0.86 μg/ml in normal adults.[9] In a previous study,[21] the mean serum IS values in patients with CKD stage 1, 2, 3 or 4 were 0.8±0.6 μg/ml, 1.0±0.6 μg/ml, 1.3±0.7 μg/ml and 4.0±3.5 μg/ml, respectively. Barreto et al reported that serum IS levels were 8.8±9.8 μg/ml in patients with CKD stage 2 to 5D.[12] In the present study, serum IS levels in the patients with kidney function equivalent to CKD stage 1, 2 and 3 were 0.55±0.53 μg/ml, 0.92±0.53 μg/ml and 1.41±0.78 μg/ml, respectively. The cut-off value 0.9 μg/ml in the present study was almost the mean value of the patients with kidney function

equivalent to CKD stage 2. We considered that the main reason for the difference between studies was our exclusion of patients with eGFR $<30\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$. Because eGFR is inversely associated with IS level,[12] patients with eGFR $<30\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ would have been expected to have high IS levels. In addition, patients in the present study showed an average LVEF of 32.5%, which indicates relatively mild LV systolic dysfunction in these DCM patients. Furthermore, almost all our subjects were in New York Heart Association functional class I or II, indicating no or mild HF symptoms.

The reason for the lack of a significant difference in cardiac death between the low and high IS groups might be the low incidence of cardiac death in the 2 groups. However, the probability of hospitalization for worsening HF was significantly higher in DCM patients with a high IS than in those with a low IS level. Furthermore, serum IS was a significant prognostic marker even after adjusting for BNP. However, neither serum creatinine nor eGFR was a significant marker (**Table 3**). Therefore, serum IS, in addition to plasma BNP, could be considered to be a more informative prognostic marker than serum creatinine and eGFR. Our findings indicate that an elevated serum IS level is associated with a poor prognosis, even in ambulatory and stable DCM patients with normal kidney function or mild to moderate CKD. In other words, the serum IS level, in addition to that of plasma BNP, could be clinically useful for assessing the severity of HF and the efficacy of its treatment. Further study is necessary to determine the therapeutic utility of IS in stable HF patients.

Association Between Serum IS and Cardiac Function

E/e' has been widely adopted as a noninvasive measure of intracardiac filling pressure.[22,23] Here, E/e' was significantly higher (ie, worse) in the high IS group than in the low IS group. Furthermore, E/e' was an independent determinant of IS. Although many studies have investigated uremic toxins, the effects of IS on the heart have rarely been evaluated. Therefore, the precise mechanism behind these effects remains unknown. Our results suggest that elevated serum IS might reflect diastolic dysfunction in patients with DCM. Further hemodynamic studies are warranted to clarify the mechanism.

QRS duration is an indication for biventricular pacing.[24,25] In our study, QRS duration was longer in the high IS group than the low IS group, and there were 3 patients with biventricular pacing in the high IS group, and none in the low IS group (**Table 1**). Although QRS duration significantly correlated with IS, it was not significantly independently associated with IS in the multivariate analysis. Our finding that a wide QRS was an important sign of poor prognosis concurs with several reports of an association between QRS duration and prognosis.[25,26] The results of our study suggest that IS might be associated with cardiac conduction disturbance, which would suggest an association between IS and progression of HF that may then require implantable cardioverter defibrillator or cardiac resynchronization therapy, or lead to cardiac events.

Toxic Effects of IS

Recent research has revealed how IS exerts its toxic biologic effects. It inhibits endothelial proliferation and wound repair,[27] and in rats it stimulates the proliferation of vascular smooth muscle cells.[19] We have previously demonstrated that IS promotes aortic calcification and aortic wall thickening in hypertensive rats.[28] Furthermore, IS has pro-fibrotic, pro-hypertrophic, and pro-inflammatory effects, indicating that it might play an important role in adverse cardiac remodeling mediated via activation of the p38 MAPK, p42/44 MAPK, and NF-κB pathways.[20,29,30] With respect to the cardiac effect of IS, Lekawanvijit et al showed that IS at concentrations ranging from 3 to 200 μmol/L significantly stimulated neonatal cardiac fibroblasts collagen synthesis in vitro.[30] In the present study, the mean serum IS level was 1.04 (μg/ml), which is equivalent to 4.88 μmol/L. Taking all the findings together, IS at the mean level observed in the present study may directly induce detrimental effects on cardiac cellular function. Further investigation to elucidate the mechanism is warranted.

Prevention of the Progression of HF

An oral sorbent (AST-120) reduces the serum and urine levels of IS in uremic rats and patients with CKD stage 4/5 by adsorbing indole in the intestines, and consequently stimulating its excretion into feces. AST-120 delays the progression of CKD not only in uremic rats, but also in CKD patients.[31–39] AST-120 is widely used as an approved drug, not only in Japan, but also in Korea, for the treatment of predialysis patients with CKD stage 4/5 to delay the progression of CKD. A multicenter, randomized, double-blind, placebo-controlled dose-ranging study has demonstrated that AST-120 decreases serum IS levels in a dose-dependent fashion in patients with CKD stage 4/5.[40] Recently, AST-120 has been reported to prevent the development of LV concentric change in predialysis CKD patients.[41] Thus, AST-120 may also be effective in preventing cardiac dysfunction by reducing the serum levels of IS.

Study Limitations

First, the small sample size and low occurrence of cardiac events limited the power to prove a relationship between serum IS and prognosis. Further studies with a larger number of patients, including those with more severe HF symptoms, are needed to confirm our results. Second, patients with advanced CKD (stages 4 and 5) and/or ischemic heart disease were excluded, so it is unclear how their inclusion would affect the results. Third, we did not investigate the mechanism of IS-induced cardiac effects. Further investigation is warranted to elucidate the mechanism.

Conclusions

Our results suggest that high IS levels are associated with cardiac dysfunction, especially diastolic dysfunction, and consequently increase the risk of cardiac events in patients with DCM. Further clinical studies with a larger number of both subjects and cardiac events are required to confirm our findings.

Disclosures

Conflicts of Interest: None of the authors have a real or perceived conflict of interest regarding the work presented in the manuscript. Funding/Support: None.

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