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Report

Different Correlation between Serum Levels of Indoxyl Sulfate and Estimated GFR in the Elderly with or without Dementia

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A decrease in renal function leads to the accumulation of various uremic toxins (UTs) which exert unfavorable physiological effects on the body. Indoxyl sulfate (IS), a tryptophan-derived UT, is known to closely associate with the progression of cognitive disorders (CD) including dementia, in addition to chronic kidney disease (CKD) and cardiovascular events. It is, therefore, important to assess blood IS levels in CKD patients with CD. In this study, we assayed serum IS levels in 37 residents who had been admitted to a geriatric health services facility with stage G3b to G5 CKD and evaluated the correlation between serum IS levels and estimated glomerular filtration rate (eGFR). Eighteen out of 37 residents were considered to suffer dementia. When plotting all serum IS levels against eGFR, a weak but significant correlation was observed with a regression coefficient (r) of -0.420. In the non-dementia group, the correlation between serum IS levels and eGFR became stronger (r = -0.720). However, no correlation was observed in the dementia group. At CKD stage G3b, mean serum IS level was higher in the dementia group than in the non-dementia group. These results suggest that eGFR becomes a good marker to predict serum IS level in the case of CKD patients without dementia, but not in those with dementia. Therefore, direct monitoring of serum IS level is essential to assess the onset and/or progression of dementia in the elderly, irrespective of CKD stages.

Key words indoxyl sulfate, serum levels, dementia, chronic kidney disease, aged people

INTRODUCTION

Cardiovascular events (CVE) are frequent and sometimes lethal in the patients at the end-stage of chronic kidney disease (CKD).[1)] In addition, it is known that the risk for developing cognitive disorders (CD) including dementia is high in these populations.[2–4)] It is also suggested that uremic toxins (UTs) are involved in the development of CD.[5)] A variety of UTs accumulate in the blood of the patients with seriously impaired renal function.[6,7)] Indoxyl sulfate (IS) is one of the most studied UTs and its blood levels elevate markedly at the end stage of CKD.[7)] When renal function is normal, IS is efficiently excreted in the urine, being mediated by organic anion transporters, which are expressed on the luminal or abluminal membrane of renal proximal tubular cells.[8–11)] As IS is a highly protein-bound UT,[12)] the contribution of glomerular filtration to the urinary excretion of IS is limited. Therefore, the elevation of blood IS levels in CKD patients mostly reflects the decreased function of renal secretory systems.

Greater IS accumulation in the cerebrospinal fluid (CSF) had long been known in the uremic patients.[13)] Very recently, Sankowski *et al.*[14)] reported a greater IS accumulation in CSF obtained from the patients with Parkinson's disease. They also showed that IS levels in CSF correlated with estimated glomerular filtration rate (eGFR) in the patients with Parkinson's disease. Although the mechanism by which IS enters into the brain has not yet been clarified, it is reasonable to consider that IS accumulation in the brain is closely associated with the elevated blood IS levels.

The increase in the number of patients with dementia is serious concern in Japan now. It is known that CD appears at the early stage of CKD.[15)] Therefore, it is an important issue to collect information about the cerebro-renal interactions through IS in CKD patients. However, few reports are available about the relationship between blood IS levels and the CKD stage in elderly Japanese with dementia. The objective of this study was to assess the differences in the relationship between serum IS levels and eGFR in the elderly with or without dementia.

MATERIALS AND METHODS

Materials IS was obtained from Sigma Aldrich Co. Ltd. (St. Louis, MO, USA). Other reagents were purchased from Wako (Osaka, Japan) at the highest grade available.

Population The purpose of this study and the protection of personal information were explained orally and by written document to the residents of Hokuseikan (a geriatric health services facility belonging to Izumikai) in Chitose City, Japan. When a resident had difficulty in understanding, the explanation was provided to a family member instead. From those who agreed to participate in this study, 37 residents (male

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Table 1. Clinical Characteristics of Study Populations

	CKD Stage											
	G3b to 5 eGFR (<45)				G3b eGFR (30~44)			G4 eGFR (15~29)			G5 eGFR (<15)	
	all	non-D	D	P	non-D	D	P	non-D	D	P	non-D	D
Number	37	19	18		8	14		7	4		4	0
Age (years)	89.9 ± 1.1	89.7 ± 17	90.1 ± 1.4	0.573	89.0 ± 2.4	88.9 ± 1.6	0.959	87.6 ± 3.1	94.3 ± 2.1	0.165	95.0 ± 3.5	
Male/Female	7/30	5/14	2/16		3/5	2/12		2/5	0/4		0/4	
Scr (mg/dL)	1.56 ± 0.12	1.87 ± 0.21	1.23 ± 0.08	0.168	1.23 ± 0.08	1.10 ± 0.04	0.123	1.87 ± 0.21	1.69 ± 0.24	0.597	3.14 ± 0.49	
eGFR (mL/min /1.73 m^2)	30.3 ± 1.7	26.2 ± 2.4	34.6 ± 1.9	0.106	36.2 ± 1.5	37.8 ± 1.2	0.425	23.0 ± 1.7	23.3 ± 3.2	0.920	11.9 ± 1.6	
BUN (mg/dL)	28.9 ± 2.0	33.4 ± 3.2	24.2 ± 2.0	0.168	23.2 ± 1.9	22.0 ± 1.8	0.676	32.4 ± 2.3	32.0 ± 5.1	0.933	55.5 ± 5.0	
BW (kg)	48.1 ± 1.7	50.4 ± 2.8	45.7 ± 1.8	0.169	51.3 ± 5.0	44.1 ± 1.5	0.111	54.6 ± 4.0	51.0 ± 6.1	0.621	41.3 ± 2.0	
IS (μM)	17.1 ± 2.2	18.4 ± 3.4	15.8 ± 2.8	0.900	9.3 ± 2.4	16.6 ± 3.5	0.180	16.8 ± 2.5	12.7 ± 3.7	0.368	38.9 ± 9.1	

The values represent the mean ± S.E. P means p value between dementia (D) and non-dementia (non-D).
Scr: serum creatinine, BUN: blood urea nitrogen, BW: body weight, eGFR: estimated glomerular filtration rate, IS: indoxyl sulfate

7; female 30) with an eGFR of less than 45 mL/min/1.73 m^2 were enrolled in this study; that is, all participants in this study were regarded as CKD patients. Their average age was 89.9 (76-101). Laboratory test values such as Scr, BUN, and eGFR were checked from a review of their medical records (Table 1). Mean value of eGFR was 30.3±1.7 mL/min/1.73 m^2 with a range of 7.5 to 44.5. No participants received spherical adsorptive carbon. Also, no one underwent hemodialysis. According to their medical records, 18 of the 37 residents were regarded as suffering dementia at the time of enrollment in this study. The distribution of residents belonging to the dementia and non-dementia groups is shown in Table 1, according to three CKD stages.

Collection of Blood Samples Additional blood samples (about 5 mL) were taken when the residents received regular blood tests. Collection of blood sample was performed from October, 2017 to February, 2018, and the serum obtained was preserved at -30°C until assay.

Assay of Serum IS Levels The thawed serum (50 μL) was mixed with an equal volume of saline and 200 μL of methanol, let stand for 10 min in iced water, then centrifuged at 5,350 g for 10 min at 5°C. The IS levels in the supernatant fluid were determined by HPLC, according to assay conditions as described previously,[16] with less than 5% of the coefficient of variation.

Statistical Analyses The correlations between serum IS levels and eGFR were evaluated using Excel Statistics 2012 (Social Survey Research Information Co., Tokyo, Japan). The difference in mean serum IS level was judged by Dunnett test or Student t-test. In the statistical analyses, $p<0.05$ was considered significant.

Ethics This study was carried out under the approval of the Ethics Committee of Izumikai Medical Corporation (Approval No. 54).

RESULTS

Correlation between Serum IS Levels and eGFR in Dementia and Non-dementia Groups The mean serum IS level obtained from the 37 residents who participated in this study was 17.1±2.2 μM. The maximum and minimum levels were 65.5 μM and 2.6 μM, respectively, with a wide variation. When plotting the serum IS levels of these 37 residents against their eGFR, there was a weak but significant correlation (r = -0.420, $p<0.01$) (Fig. 1A). When the correlations between serum IS levels and eGFR were evaluated by dividing them into dementia and non-dementia groups, a much better correlation was obtained (r = -0.720, $p<0.001$) in the case of the non-dementia group (Fig. 1B), compared with that in Fig. 1A. In the case of the dementia group, however, there was no correlation between serum IS levels and eGFR (Fig. 1C). Reflecting the correlation shown in Fig. 1B, there was a positive and significant correlation between serum IS levels and serum creatinine levels in the non-dementia group. However, no correlation was observed in the dementia group (data not shown).

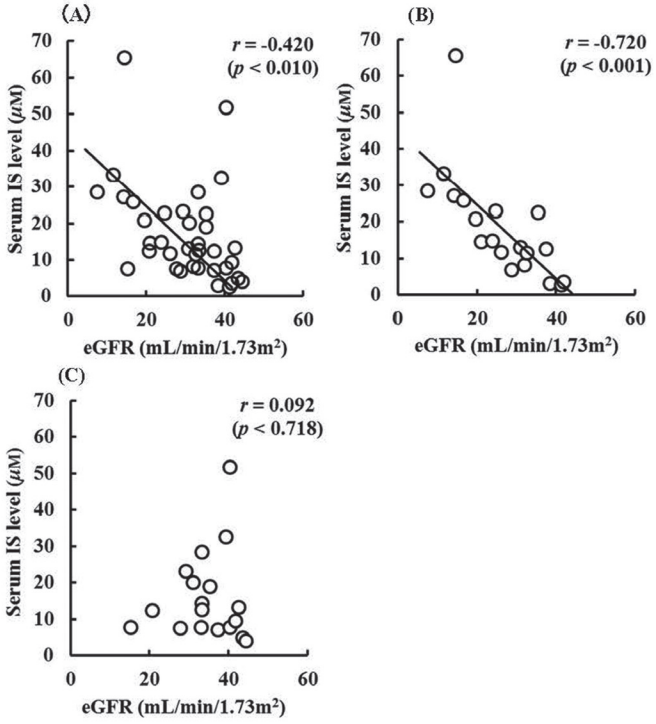

Fig. 1. Correlation between Serum IS Levels and eGFR in All 37 Residents (A), in Non-dementia Group (B), and in Dementia Group (C)

Each point represents the individual value obtained from the 37 residents.

Comparison of Serum IS Levels between Dementia and Non-Dementia Groups As a whole, no significant difference was observed in the mean serum IS levels between dementia and non-dementia groups (Fig. 2). However, when comparing serum IS levels at each CKD stage, there was a tendency for the mean serum IS level in the dementia group to

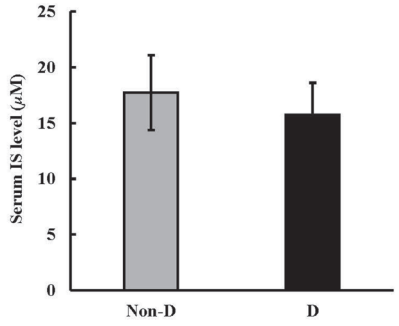

Fig. 2. Comparison of Mean Serum IS Levels between Dementia and Non-dementia Groups

Data represents the mean ± S.E. obtained from 18 residents with dementia and 19 residents without dementia. There was no significant difference between these two groups. D means dementia.

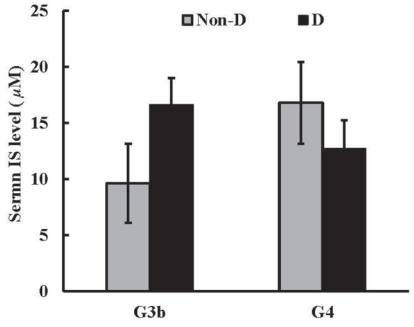

Fig. 3. Comparison of Mean Serum IS Levels between Residents with or without Dementia at CKD G3b and G4

Data represents the mean ± S.E. obtained from 18 residents with dementia and 15 residents without dementia. D means dementia.

increase beyond that in the non-dementia group in the case of G3b, although the difference was not significant (Fig. 3). The tendency was not evident in the case of G4.

DISCUSSION

IS accumulation in the blood of CKD patients becomes a trigger for further progression of CKD and the occurrence of CVE.[17–19] IS is also considered as a biomarker associated with the reduced renal function in CKD patients.[20] Accordingly, many interests have been placed on the blood IS levels in CKD patients and it has been suggested that eGFR is a useful parameter to roughly know blood IS levels.[21,22] However, little information is available about how the relationship between eGFR and serum IS levels changes in CKD patients when they suffer from various complications. It is well known that CD including dementia is frequent in CKD patients. In this study, therefore, we investigated the correlation between eGFR and serum IS levels in the elderly with CKD and dementia. According to previous results, it is assumed that accumulation of IS and creatinine in the blood of CKD patients takes place in parallel. In order to access this, we first compared the correlation between serum IS levels and eGFR between the elderly with or without dementia. Consistent with previous studies,[21,22] there was a significant correlation between serum IS levels of 37 residents and their eGFR (Fig.1A). However, the *r* value was at most -0.420, indicating that the correlation between them was not so relevant. When the relationship between serum IS level and eGFR was evaluated in dementia and non-dementia groups separately, a better correlation was obtained in the non-dementia group (Fig. 1B), showing that eGFR is a good marker to access serum IS levels in the non-dementia group. In the case of the dementia group, however, serum IS levels did not correlate with eGFR at all (Fig. 1C), implying that eGFR is not useful for elucidating serum IS levels in the elderly with both CKD and dementia. These results suggested the relationship between serum levels of IS and creatinine in CKD patients changes extremely in the presence or absence of dementia. As far as we know, this is a new information about the relationship between IS and creatinine in the elderly with dementia. In this study, the age of the residents ranged from 76 to 101. Thus, it is important to evaluate the effect of age on the relationship between serum IS levels and eGFR. As the number of residents was not enough to do it, further approach is now undertaken by recruiting additional participants.

Recently, there has been an increased interest in the pathogenic relationship between colonic microbiota and CKD in terms of the gut-kidney axis.[23] It has been shown that the metabolism from tryptophan to indole, the precursor of IS, is enhanced in the colon of patients with CKD.[24–26] This is attributable in part to the increased presence of tryptophanase, an enzyme involved in the metabolic process of tryptophan and produced by microbiota, as shown in CKD rats.[27] Moreover, tryptophan absorption in the small intestine decreases markedly in the patients with dementia.[28] This tryptophan malabsorption leads to the further production of indole in CKD patients with dementia, followed by efficient metabolism to IS in the liver. Accordingly, it is expected that serum IS levels elevated to a greater extent in the residents with dementia than in those without dementia. As shown in Fig. 2, however, there was no difference in the serum IS levels between dementia and non-dementia groups among total 37 residents. On the other hand, while the mean serum IS levels were almost identical at CKD stage G4 (Fig. 3), it was higher in the dementia group than in the non-dementia group at CKD stage G3b, although the difference was not significant possibly due to a small number of participants (Fig. 3). These results were consistent with previous results reported by Yeh *el*.[29] They suggested that a higher serum IS level was associated with CD in the early-stage of CKD. The reason for the discrepancy between CKD stage 3 and 4 observed in this study is currently unclear.

It is thought that IS entry into the brain could be enhanced through its elevated blood levels in CKD patients with dementia. It is probable that the elevation of IS levels in blood is partially related to polymorphism of OAT3[30] or interference with OAT-mediated transport system by other UTs or concomitant drugs taking for a long time in CKD patients with dementia. Further study is required to clarify these possibilities. IS induces oxidative stress. Therefore, if IS accumulation in the brain becomes a trigger of the onset and/or progression of dementia, direct monitoring of blood IS levels is essential for taking measure against dementia in the elderly. Further study is needed to get more information about blood IS levels in the elderly with or without dementia.

In conclusion, although this study was carried out in the small scale, our present results suggested that the elevation of serum IS levels in the elderly with dementia occurs without connecting to the decrease in the renal function. Thus, for the elderly showing relatively higher blood IS levels even in the

early stage of CKD, administration of drugs such as spherical adsorptive carbon should be considered to lower blood IS levels.

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Conflict of interest The authors declare no conflict of interest.

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