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Association between The Accumulation of Phenyl Sulfate and Macrocytosis in Hemodialysis Patients

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Abstract

Background：Protein fermentation by intestinal bacteria results in the formation of several uremic solutes. Phenyl sulfate（PHS）and *p*-cresyl sulfate（PCS）are well-known gut-derived uremic solutes. Macrocytosis is frequently observed in hemodialysis patients, and the association of macrocytosis with mortality has been reported in these patients. We investigated the association between serum PHS level and macrocytosis in hemodialysis patients and evaluated the factors determining PHS accumulation in the blood.

Patients and methods：We surveyed the diet and bowel habits of 28 patients with hemodialysis（20 men, 8 women）, and measured PHS and PCS serum levels by high performance liquid chromatography. Macrocytosis was defined as 100 fL≤mean corpuscular volume（MCV）.

Results：Nine subjects（32%）showed macrocytosis, while the remainder（68%）were normocytic（80 fL≤MCV＜100 fL）. The serum PHS level in macrocytic subjects was significantly higher（$P=0.019$）than that in normocytic subjects. Serum PHS correlated with the ratio of protein intake to dietary fiber intake（protein-fiber index；rs＝－0.42, $P=0.029$）and defecation frequency（rs＝0.38, $P=0.047$）. These results

were contrary to a correlation with the serum PCS and the protein-fiber index（rs＝0.41, P＝0.034）and defecation frequency（rs＝－0.47, P＝0.011）. Serum PCS levels were not significantly different between macrocytic and normocytic subjects.

Conclusion：Increased serum PHS levels may be associated with the development of macrocytosis in hemodialysis patients. The relationship between serum PHS and diet or bowel habits may be different from that of PCS.

Introduction

Renal anemia, developed in patients with moderate kidney dysfunction, affects the clinical condition of chronic kidney disease (CKD). Although anemia in CKD is typically normocytic, macrocytosis is occasionally observed in CKD patients with maintenance hemodialysis[1]. Macrocytosis, defined as a high mean corpuscular volume（MCV）, may be associated with mortality in hemodialysis patients[1]. Recently, MCV has been associated with mortality in stage 3-5 CKD patients[2]. The decline in erythrocyte production-stimulating hormone produced by the kidney causes mainly renal anemia. In other cases, chronic inflammation, iron deficiency, and a shortened red blood cell half-life are exacerbation factors of anemia in CKD[3]. Several uremic toxins were also reported to cause anemia[4)-6)]. However, research on the participation of uremic toxins in macrocytosis is insufficient.

Products from protein fermentation by intestinal bacteria cause uremic solutes. Phenyl sulfate（PHS）and *p*-cresyl sulfate（PCS）are known gut-derived phenolic uremic solutes. Serum PCS levels are associated with cardiovascular disease and mortality in elderly hemodialysis patients[7]. Although PHS accumulation is detected in CKD patients, the association between serum PHS levels and the development of complications is unclear. PHS and PCS are sulfo-conjugate metabolites of phenol and *p*-cresol, respectively. Two precursors are synthesized from tyrosine, which is a common substrate used by intestinal bacteria. Since the tyrosine source is protein escaped from intestinal digestion and absorption, the production of PHS and PCS is influenced by diet. Furthermore, we previously indicated an association between total serum *p*-cresol levels and bowel habits[8].

We clarified the association of serum phenolic uremic solute levels with macrocytosis in hemodialysis patients. We surveyed diet and bowel habits, and compared the factors influencing PHS and PCS accumulation in the blood.

I Patients and Methods

1. Patients

This study was performed on 33 outpatients undergoing hemodialysis three times a week at two outpatient hemodialysis clinics（Toyoda Clinic and Hachioji Kidney Clinic）. The selection criteria for the subjects were men and women between the ages of 35 and 80 years. Written informed consent was obtained from all subjects before study participation. The ethics committee of the Tokyo Medical University approved this study（No. 1734）.

2. Study design

The study duration was 4 weeks. Subjects were instructed to eat their usual meals, and avoid any supplemental foods and drugs containing probiotics, prebiotics, and dietary fibers during the study period. Subject background data (such as age, primary disease, medications taken, and drugs used during hemodialysis) were collected. Two weeks after the study start, subjects filled out a questionnaire regarding defecation every day for 2 weeks. Additionally, the diet survey was performed on the last 3 days, and blood was collected on the last day of the study. Blood collection was unified at the third day from the last hemodialysis.

We defined microcytosis, normocytosis, and macrocytosis as MCV＜80 fL, 80 fL≤MCV＜100 fL, and 100 fL≤MCV, respectively. No microcytic subjects were observed. We divided the study group into macrocytic and normocytic subjects, and we compared laboratory data, diet, and bowel habits between these groups.

3. Laboratory analyses

PHS and PCS were purchased from Tokyo Chemical Industry (Tokyo, Japan). Serum PHS and PCS levels were analyzed by high performance liquid chromatography (GL-7400；GL Sciences, Tokyo, Japan). Briefly, serum was added to three volumes of methanol, and deproteinized[9]. The ODS-SP column (4.6×150 mm, 5 μm particle size；GL Sciences) with a guard column (4.0×10 mm) was maintained at 40℃. The mobile phases consisted of 0.02 M phosphate-buffer (pH 4.0) (A) and methanol (B) with a gradient from A92%/B8% (0 min) to A78%/B22% (21 min). The flow rate was 1.0 mL/min. PHS and PCS were detected by fluorescence with excitation/emission at 214/306 nm. The detection limits of PHS and PCS were 0.9 and 0.4 mg/L, respectively.

Serum indoxyl sulfate was analyzed as described previously[8]. Blood urea nitrogen, creatinine, intact parathyroid hormone (iPTH), β_2-microgloblin, red blood cell (RBC), hemoglobin, MCV, mean corpuscular hemoglobin concentration (MCHC), total iron binding capacity, unsaturated iron binding capacity, serum iron (Fe), ferritin, and transferrin saturation (TSAT) were measured using standard methods.

4. Survey of diet and bowel habits

A registered dietitian estimated the nutritional values of dietary intake (energy, protein, fat, carbohydrate, and dietary fiber) based on meal records and photographs. Additionally, the daily energy intake, protein per ideal body weight (IBW), and ratio of protein intake to dietary fiber intake (protein-fiber index)[10] were calculated.

Subjects were asked to evaluate, via a questionnaire, their defecation frequency (total count per 2 weeks). The stool form was scored for each defecation with the Bristol stool form scale[11], and was calculated as the average of scores per defecation.

5. Statistical analysis

Bowel habit data were expressed as median (25%, 75%). Other data were expressed as mean±S.D. for normal or median (25%, 75%) for non-normal distributions. The Shapiro-Wilk test was used to assess the normal distribution. Fisher's exact test or the Mann-Whitney U test were used to compare the macrocytic and normocytic subjects. Additionally, defecation frequency and PHS were compared between subjects who did or did not use laxatives. The corre-

lation between serum PHS or PCS levels and other items were evaluated by Spearman's rank correlation coefficient. $P<0.05$ was considered statistically significant. SPSS Version 11 (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses.

Ⅱ Results

1. Subjects

During the study, two subjects discontinued participation due to hospitalization and one did so according to their own decision. Two patients were excluded as they were treated with drugs containing live bacteria during the study. Finally, data from 28 subjects (20 men and 8 women) were analyzed. Their primary diseases were chronic glomerulonephritis ($n=10$), diabetic nephropathy ($n=9$), nephrosclerosis ($n=3$), and others ($n=6$). **Table 1** shows subject characteristics. Data from one subject were excluded due to incomplete diet and bowel habit records. Nine subjects (32%) showed macrocytosis. Nineteen subjects (68%) were normocytic.

2. Laboratory data

Serum PHS and PCS levels in all subjects ranged from 2.3-35.8 and 0.4-90.5 mg/L, respectively. Hemoglobin concentration and MCV ranged from 9.4-12.3 g/dL and 83.8-105.2 fL, respectively. Six patients had ferritin levels<100 ng/mL and TSAT<20%.

Serum PHS levels were higher in macrocytic subjects than in normocytic ones ($P=0.019$), and RBC levels were lower in the former ($P<0.001$). Hemoglobin concentration did not differ between groups. The MCHC of all subjects was in the normal range (32-36%), although it was higher in macrocytic subjects ($P=0.002$). Fe and TSAT values in macrocytic subjects ($P=0.036$) were higher than those in normocytic ones ($P=0.028$). The serum PCS level, and levels of three uremic toxins (iPTH, β_2-microglobrin, and indoxyl sulfate) did not differ between the groups (**Table 1**).

The serum PHS level negatively correlated with RBC ($P=0.004$), and positively correlated with MCV ($P=0.019$), MCHC ($P=0.050$), and TSAT ($P=0.022$). Serum PCS levels did not correlate with any item (**Table 2**).

The correlation coefficients (P values) of iPTH, β_2-microglobulin, and indoxyl sulfate with hemoglobin concentration were −0.01 (0.944), 0.21 (0.291), and −0.28 (0.155), respectively. The levels of PHS, PCS, and these uremic toxins did not correlate with hemoglobin concentration.

3. Nutritional intake

The daily intake of energy and protein per IBW in all subjects was 25.6 kcal/kg/day and 0.91 g/kg/day, respectively. Energy intake was below the clinically recommended range (30-35 kcal/kg/day) for CKD in Japan, and protein intake was in the minimum recommended range (0.9-1.2 g/kg/day)[12]. Dietary fiber intake (10.2 g/day) in all subjects was similar to that in a previous report of hemodialysis patients[13]. The dietary fiber intake was much lower than the Dietary Reference Intake for dietary fiber at ages 18 or older in Japanese subjects (19 g/day≤male, and 17 g/day≤female)[14]. These data indicated that our subjects had a comprehensively low intake of dietary energy, protein, and fiber. Comparing macrocytic and normocytic subjects, the daily intake of energy per IBW ($P=0.027$) and daily intake of fat ($P=0.045$) were

Table 1 Characteristics, laboratory data, nutritional intake, and bowel habits

	All ($n=28$)	MCV＜100 fL ($n=19$)	100 fL≤MCV ($n=9$)	*P*
Male/Female	20/8	14/5	6/3	0.516
Age（years）	63（54, 73）	63（51, 73）	62（56, 73）	0.882
Body mass index（kg/m^2）	21.9±3.4	22.1±3.9	21.4±2.3	0.980
Hemodialysis duration（years）	2.8（1.3, 5.6）	2.2（1.3, 5.1）	4.4（2.3, 8.3）	0.257
Medication				
ESAs（%）	86	95	67	0.084
Iron（intravenous）（%）	21	26	11	0.350
Laxatives（%）	46	37	67	0.142
Laboratory data				
BUN（mg/dL）	63±11	63±11	64±11	0.768
Creatinine（mg/dL）	10.7±2.4	10.6±2.3	10.9±2.8	0.883
iPTH（pg/mL）	187±100	198±117	163±44	0.491
β_2-microgloblin（mg/L）	25.0±6.9	25.1±7.0	24.7±7.1	0.825
Indoxyl sulfate（mg/L）	34.2±13.1	35.1±14.5	32.4±9.8	0.712
PCS（mg/L）	35.5±20.7	36.6±22.3	33.2±17.9	0.863
PHS（mg/L）	9.3（6.1, 14.8）	6.5（5.3, 10.8）	14.8（9.0, 18.8）	0.019
RBC（×10^4/μL）	341±35	356±31	310±21	＜0.001
Hemoglobin（g/dL）	10.9±0.7	11.0±0.7	10.7±0.6	0.538
MCV（fL）	96.7±5.1	94.2±4.2	102.0±1.7	＜0.001
MCHC（%）	33.1±0.8	32.8±0.7	33.8±0.5	0.002
Fe（μg/dL）	70±20	64±20	82±16	0.036
TIBC（μg/dL）	262±43	266±47	253±35	0.363
UIBC（μg/dL）	192±46	202±49	171±32	0.081
Ferritin（ng/mL）	69（30, 120）	62（23, 118）	76（49, 119）	0.538
TSAT（%）	27±8	25±8	33±7	0.028
Nutritional intake（per day），$n=27$				
Energy（kcal）	1486±390	1396±373	1666±379	0.064
（kcal/kg IBW）	25.6±6.5	24.2±6.8	28.5±5.2	0.027
Protein（g）	53±15	52±14	54±19	0.504
（g/kg IBW）	0.91±0.27	0.90±0.24	0.93±0.33	0.643
Fat（g）	47（36, 58）	38（35, 52）	57（50, 59）	0.045
Carbohydrate（g）	206±56	194±54	228±55	0.150
Fiber（g）	10.2（8.6, 12.6）	10.3（8.4, 12.7）	10.0（9.9, 11.6）	0.719
Protein-fiber index	5.3±2.0	5.5±2.0	5.0±2.0	0.537

（continued）

(continued)

	All ($n=28$)	MCV＜100 fL ($n=19$)	100 fL≤MCV ($n=9$)	P
Bowel habits				
Defecation (times/2 weeks)	16 (12, 21)	15 (12, 19)	21 (14, 22)	0.126
Stool form (score 1-7)	4.3 (4.0, 5.0)	4.2 (3.9, 4.7)	4.7 (4.2, 5.0)	0.201

Values are expressed as Median (25%, 75%) or Mean±S.D. and P-value (Fisher exact test or Mann-Whitney U test ; MCV＜100 fL group vs. 100 fL≤MCV group)
ESAs ; erythropoiesis stimulating agents, BUN ; blood urea nitrogen, iPTH ; intact parathyroid hormone, PHS ; phenyl sulfate, PCS ; p-cresyl sulfate, RBC ; red blood cell, MCV ; mean corpuscular volume, MCHC ; mean corpuscular hemoglobin concentration, IBW ; ideal body weight, Fe ; serum iron, TIBC ; total iron binding capacity, UIBC ; unsaturated iron binding capacity, TSAT ; transferrin saturation, Protein-fiber index ; ratio of protein intake to dietary fiber intake

Blood was collected on the last day of the study. Blood collection was unified at the third day from the last hemodialysis. Serum indoxyl sulfate, PHS, and PCS levels were analyzed by high performance liquid chromatography. BUN, creatinine, iPTH, β_2-microgloblin, RBC, hemoglobin, MCV, MCHC, TIBC, UIBC, Fe, ferritin, and TSAT were measured using standard methods.

The diet survey was performed on the last 3 days. A registered dietitian estimated the nutritional values of dietary intake based on meal records and photographs. Daily energy intake, protein per ideal body weight, and ratio of protein intake to dietary fiber intake were calculated.

Two weeks after the study start, subjects filled out a questionnaire regarding defecation every day for 2 weeks. Subjects were asked to evaluate, via a questionnaire, their defecation frequency (total count per 2 weeks). The stool form was scored for each defecation with the Bristol stool form scale, and was calculated as the average of scores per defecation.

higher in the macrocytic subjects. The intake of protein, carbohydrates, and dietary fiber did not differ between groups (**Table 1**).

Serum PHS level did not correlate with dietary fiber intake, although serum PCS levels showed a negative correlation (rs＝−0.43, $P=0.024$; **Table 2**). Serum PHS levels negatively correlated with the protein-fiber index [rs＝−0.42, $P=0.029$; **Table 1**, **Figure 1**-(A)], whereas serum PCS levels positively correlated with the protein-fiber index [rs＝0.41, $P=0.034$; **Table 1**, **Figure 1**-(B)].

4. Bowel habits

In all subjects, the defecation frequency was 16 (12, 21) times/2 weeks. Only one subject had a defecation frequency＜7 times/2 weeks and none had a defecation frequency ＞28 times/2 weeks. Bowel habits were not different between macrocytic and normocytic subjects (**Table 1**). In the survey of bowel habits, defecation frequency was normal.

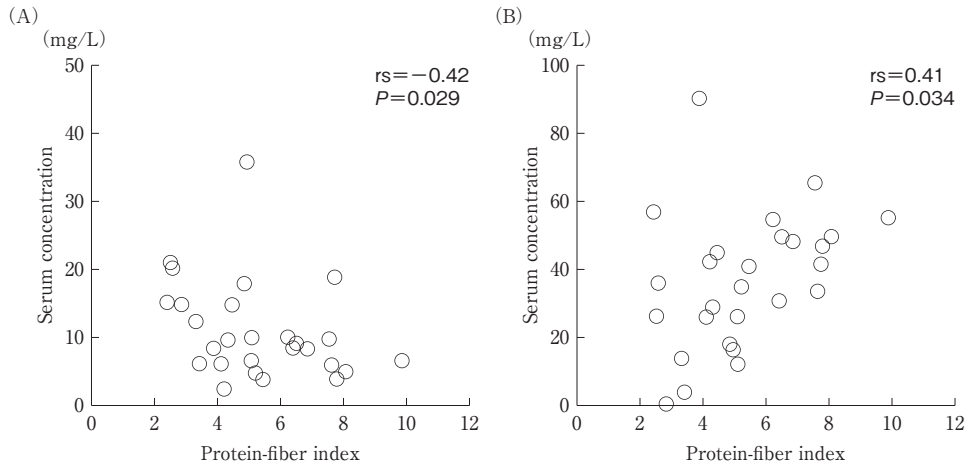
The laxative use rate (46%) was high in agreement with a previous report[8]. Hemodialysis patients tended to control defecation with laxatives. Defecation frequency was not different between those who did and did not use laxatives. Serum PHS levels in laxative users and nonusers were 12.3 (9.6, 17.9) and 6.5 (5.9, 9.3) mg/L, respectively. This difference was significant ($P=0.027$).

Serum PHS levels positively correlated with defecation frequency [rs＝0.38, $P=0.047$; **Table 2**, **Figure 2**-(A)], although serum PCS levels negatively correlated with it [rs＝−0.47, $P=0.011$; **Table 2**, **Figure 2**-(B)].

Table 2 The correlation between serum PHS or PCS levels and other items

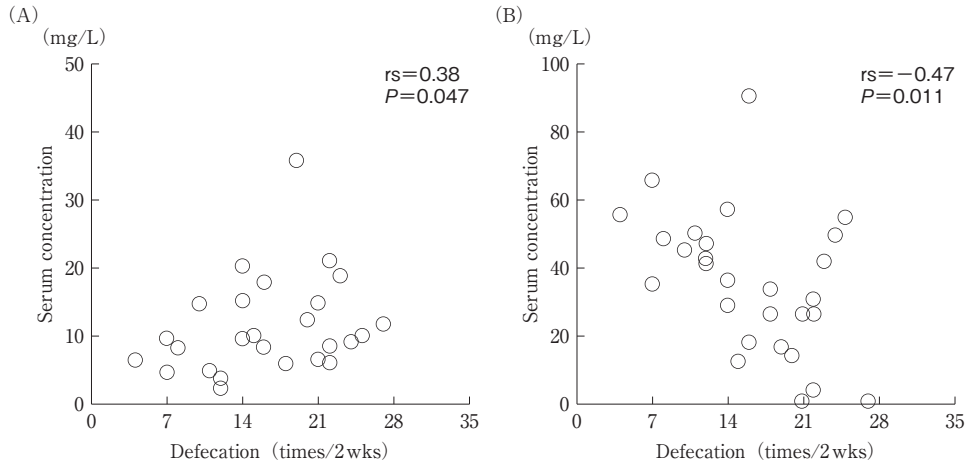
	Correlation coefficient (*P* value)	
	PHS	PCS
Age (years)	−0.00 (0.981)	−0.05 (0.782)
Body mass index (kg/m^2)	−0.20 (0.315)	−0.16 (0.418)
Hemodialysis duration (years)	0.31 (0.111)	−0.16 (0.411)
Laboratory measurements		
BUN (mg/dL)	−0.07 (0.731)	0.06 (0.766)
Creatinine (mg/dL)	0.17 (0.379)	0.12 (0.556)
iPTH (pg/mL)	0.14 (0.490)	−0.33 (0.084)
β_2-microgloblin (mg/L)	0.14 (0.473)	0.14 (0.492)
Indoxyl sulfate (mg/L)	−0.05 (0.812)	0.32 (0.100)
PCS (mg/L)	−0.24 (0.216)	− −
PHS (mg/L)	− −	− −
RBC ($\times 10^4/\mu$L)	−0.52 (0.004)	0.17 (0.386)
Hemoglobin (g/dL)	−0.26 (0.182)	0.06 (0.775)
MCV (fL)	0.44 (0.019)	−0.10 (0.616)
MCHC (%)	0.37 (0.050)	−0.30 (0.117)
Fe (μg/dL)	0.48 (0.010)	−0.33 (0.084)
TIBC (μg/dL)	−0.17 (0.381)	−0.22 (0.262)
UIBC (μg/dL)	−0.31 (0.111)	−0.08 (0.695)
Ferritin (ng/mL)	0.17 (0.396)	0.12 (0.546)
TSAT (%)	0.43 (0.022)	−0.18 (0.352)
Nutrition intake (per day)		
Energy (kcal)	−0.19 (0.352)	0.09 (0.639)
(kcal/kg IBW)	−0.07 (0.716)	0.06 (0.774)
Protein (g)	−0.36 (0.067)	0.05 (0.807)
(g/kg IBW)	−0.35 (0.073)	−0.04 (0.861)
Fat (g)	−0.19 (0.330)	−0.08 (0.678)
Carbohydrate (g)	−0.11 (0.585)	0.19 (0.337)
Fiber (g)	−0.06 (0.767)	−0.43 (0.024)
Protein-fiber index	−0.42 (0.029)	0.41 (0.034)
Bowel habits		
Defecation (times/2 weeks)	0.38 (0.047)	−0.47 (0.011)
Stool form (score 1-7)	0.05 (0.804)	−0.25 (0.201)

The correlation between serum PHS or PCS levels and other items were evaluated by Spearman's rank correlation coefficient.



The correlation between protein-fiber index and serum PHS(A) or serum PCS(B). Serum PHS levels negatively correlated with the protein-fiber index, whereas serum PCS levels positively correlated with the protein-fiber index.

Figure 1 The correlation between protein-fiber index and serum PHS or PCS levels



The correlation between defecation frequency and serum PHS(A) or serum PCS(B). Serum PHS levels positively correlated with defecation frequency, although serum PCS levels negatively correlated with it.

Figure 2 The correlation between defecation frequency and serum PHS or PCS levels

Ⅲ Discussion

We investigated the association between macrocytosis and gut-drived uremic solutes, such as PHS and PCS in hemodialysis patients. This study demonstrated that serum PHS levels in macrocytic subjects are significantly higher than those in normocytic hemodialysis patients. Moreover, a low protein-fiber index and high defecation frequency were associated with the accumulation of PHS in the blood. These findings are shown for the first time.

The Japanese guidelines for renal anemia in CKD[15] recommended values of ferritin＜100 ng/mL and TSAT＜20% as application criteria for iron supplementation. The recommended concentration of hemoglobin is 10-12 g/dL for treatment of anemia in hemodialysis patients. In this study, six patients had ferritin＜100 ng/mL and TSAT＜20%. Their hemoglobin concentrations were almost consistent with the recommended value. Iron deficiency anemia typically is microcytic and hypochromic. In our study, no microcytic subjects (MCV＜80) were observed. Thus, anemia in our subjects was appropriately controlled by iron supplementation and erythropoiesis-stimulating agents.

Bataille, et al.[16] indicated that the plasma concentrations of indoxyl sulfate and PCS did not correlate with the hemoglobin concentration. In our study, the hemoglobin concentration was not associated with PHS or other uremic toxins (iPTH, β_2-microgloblin, indoxyl sulfate, and PCS) in any subject. Our results suggested that PHS and these uremic toxins do not influence hemoglobin concentration.

In the analysis of the association between serum PHS or PCS level and MCV, the serum PHS level was higher in macrocytic than in normocytic subjects. On the other hand, the serum PCS level was not different between macrocytic and normocytic subjects. Furthermore, other uremic toxins also showed no difference. Thus, among these uremic toxins, only PHS may relate to increased MCV.

We attempted to determine some factors influencing the accumulations of PCS and PHS in blood. Regarding the PCS accumulation, high protein-fiber index and decreased dietary fiber intake were effective. A similar finding has been reported in nondialysis CKD and hemodialysis patients[10)17]. We suggested that the serum PCS level was influenced by amount of dietary fiber intake in hemodialysis patients. Regarding bowel habits in this study, the increased serum PCS level was associated with decreased defecation frequency. We previously reported an association between increased total *p*-cresol level in serum and bowel habits in hemodialysis patients[8]. Consequently, we also suggested that serum PCS level was influenced by bowel habits.

Low protein-fiber index and high defecation frequency were associated with the accumulation of PHS in the blood. There was a tendency for correlation between serum PHS and protein intake ($rs=-0.36$, $P=0.067$), while the serum PHS level did not correlate with dietary fiber intake ($rs=-0.06$, $P=0.767$; **Table 2**). The protein-fiber index was calculated as the daily protein intake per daily dietary fiber intake. Thus, PHS accumulation in blood may be influenced by decreased protein intake, but not by the increased dietary fiber intake.

The protein-fiber index and defecation frequency may have opposing effects on the serum accumulation of PHS and PCS, regardless of their production through similar mechanisms in the intestine. As one possibility, the contribution of intestinal bacteria can be considered. Many bacterial species that produce phenol and/or *p*-cresol exist in the intestine[18]. Intestinal bacteria may competitively use tyrosine, the substrate derived from proteins, in the intestine. We speculated that meal constituents and bowel conditions could change the composition of intestinal microflora, and subsequently influence phenol and *p*-cresol production.

The fact that energy and fat intake were higher in macrocytic than normocytic subjects, and that there was no difference in the intake of other nutrients in both groups indicated that macrocytic subjects had a sufficient nutrient intake compared to normocytic subjects. Thus, we concluded that nutritional factors did not directly cause macrocytosis in our study subjects.

There are two limitations to this study：1）a small sample size（28 subjects), whereby a large-scale investigation is needed to obtain unequivocal results；and 2）we lacked data on blood levels and dietary intake of vitamin B_{12} and folic acid. Generally, vitamin B_{12} and folic acid deficiency with malnutrition are associated with the development of macrocytosis[19]. It is reported that parenteral vitamin B_{12} does not change MCV in hemodialysis patients with macrocytosis[20]. A high dose folic acid supplementation normalized MCV in hemodialysis patients with macrocytic anemia[21]. Although serum PHS levels were associated with MCV, further studies are needed to clarify whether PHS is a factor in the pathogenesis of macrocytosis independent of vitamin B_{12} and folic acid.

Our results showed that, among hemodialysis patients, serum PHS level in macrocytic subjects was significantly higher than that in normocytic subjects. We believe that the increased in the serum PHS levels let to the development and progression of macrocytosis. Moreover, the serum PHS levels correlated with a low protein-fiber index and increased defecation frequency；thus, serum PHS levels may be influenced by diet and bowel habits.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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原著

血液透析患者におけるフェニル硫酸の蓄積と大赤血球症との関連

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要　約

背景：腸内細菌によるタンパク質発酵によりいくつかの尿毒症物質が生成される。フェニル硫酸（PHS）とパラクレジル硫酸（PCS）は代表的な腸由来の尿毒症物質である。血液透析患者では大赤血球症がしばしば認められ，大赤血球症と死亡率との関連が報告されている。我々は血液透析患者における血清PHSと大赤血球症との関連を検討し，さらに血清PHSの蓄積因子を評価した。

対象と方法：血液透析患者28名（男性20名，女性8名）の食習慣と排便習慣を調査し，高速液体クロマトグラフィーでPHSとPCSの血清濃度を測定した。大赤血球症は平均赤血球容積（MCV）100fL以上と定義した。

結果：対象者9名（32%）は大赤血球症であり，残り（68%）は正球性（80fL≤MCV＜100fL）であった。大球性の対象者におけるPHSの血清濃度は正球性の対象者よりも有意に高かった（$P=0.019$）。PHSの血清濃度は食物繊維摂取量に対するタンパク質摂取量の比率（タンパク質食物繊維指数）と負の相関（rs＝－0.42, $P=0.029$）および排便回数と正の相関（rs＝0.38, $P=0.047$）を示した。これらの結果はPCSの血清濃度とタンパク質食物繊維指数（rs＝0.41, $P=0.034$）および排便回数（rs＝－0.47, $P=0.011$）との相関関係とは逆であった。大球性および正球性の対象者間でPCSの血清濃度には有意な違いはなかった。

結語：PHSの血清濃度の上昇は血液透析患者の大赤血球症の発症に関連している可能性がある。PHSの血清濃度と食事または排便習慣との関係はPCSのそれとは異なる可能性がある。

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