BPB Reports

Regular Article

Association between Whole Blood Manganese and Dry Skin in Hemodialysis Patients

Masataka Deguchi,[a,b] Hirofumi Machida,[c] Hiroyuki Yasui,[d] Jun Hiraoka,[d] Keigo Nishida,[*,e] Keiichi Hiramoto,[f] Hidehiko Jose,[g] Misao Takeuchi,[c] and Kazuya Ooi[*,a]

[a]*Laboratory of Clinical Pharmacology, Graduate School of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki, Suzuka, Mie 513-8670, Japan;* [b]*Medical Link Inc., 2-17, Ureshino-Nakagawa Shinmachi, Matsusaka, Mie 515-2325, Japan;* [c]*Shoujunkai Takeuchi Hospital, 82 Kitamarunouchi, Tsu, Mie 514-0031, Japan;* [d]*Department of Analytical & Bioinorganic Chemistry, Division of Analytical & Physical Sciences, Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina, Kyoto 607-8414, Japan;* [e]*Laboratory of Immune Regulation, Graduate School of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki, Suzuka, Mie 513-8670, Japan;* [f]*Laboratory of Pathophysiology and Pharmacotherapy, Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki, Suzuka, Mie 513-8670, Japan;* [g]*Jose Clinic, 472-4 Shinden, Odai, Mie 519-2423, Japan*

Received July 18, 2021; Accepted July 20, 2021

Background and aim: Abnormalities in blood metal concentrations and pruritus occur frequently in hemodialysis patients. Pruritus significantly impairs the quality of life for these patients, and may be related to abnormal blood metal concentrations. Therefore, we measured the blood metal concentrations in hemodialysis patients and non-dialysis control patients to examine the relationship between blood metal level, pruritus, and skin condition. Methods: Hemodialysis patients were divided into "scratching" and "non-scratching" groups based on the severity of their symptoms. Blood was collected and the skin condition was measured at the start of hemodialysis in the patient group. Concentrations of metals such as magnesium, calcium, manganese, iron, copper, and zinc in serum and whole blood were determined by an inductively coupled plasma-mass spectrometer. Skin condition was assessed by measuring transepidermal water loss and stratum corneum moisture content. Results: The whole blood manganese level in hemodialysis patients was 8 times higher than that in non-dialysis patients (168 ± 42 ng/mL vs 22 ± 15 ng/mL), and a negative correlation was found between manganese level and stratum corneum moisture content. The stratum corneum moisture content in the scratching group was significantly lower than that in the non-scratching group. Conclusion: Patients with higher levels of whole blood manganese exhibited dry skin.

Key words hemodialysis, manganese, pruritus, dry skin, blood metal, inductively coupled plasma-mass spectrometer

INTRODUCTION

There are approximately 330,000 hemodialysis patients in Japan, accounting for more than one in 400 individuals, and the number is increasing every year.[1] Many hemodialysis patients have serious constraints in daily life including treatment that requires several hours every other day, and the need to adhere to strict diet and fluid restrictions. In addition, hemodialysis patients often suffer from complications including hypertension, anemia, osteoporosis, and pruritus accompanied by dry skin. Studies have reported that pruritus occurs in at least 80% of hemodialysis patients.[2–4] In addition, moderate to severe pruritus causes sleep problems and affects mortality.[4,5] Therefore, treating pruritus is critical to improving the patient's QOL.

The pathophysiology of pruritus in hemodialysis patients has yet to be completely understood, but several factors are likely to be involved in its occurrence. Recent hypotheses have suggested that changes in immunity and the opioid system cause pruritus.[6] Other factors include the development of secondary hyperparathyroidism, increase in blood urea nitrogen, calcium, phosphorus, aluminum, magnesium, β2-macroglobulin, ferritin, vitamin A, and white blood cells, decrease in erythropoietin, transferrin and albumin, anemia, increase in chemical mediators released from mast cells, and dry skin caused by atrophy and dehydration of sweat glands.[7,8] Hemodialysis patients exhibit a variety of dermatological symptoms. Oral antihistamines, topical steroids, moisturizers and the like have been commonly used for the treatment of pruritus. In 2009, nalfurafine hydrochloride, a κ-opioid receptor agonist that has a therapeutic effect on pruritus caused by central factors, was launched in Japan, expanding the range of treatment options. Medicines such as gabapentin and pregabalin may also be effective on pruritus caused by central factors.[9,10] Although various treatment strategies have been proposed, they are ineffective for many patients, and satisfaction with treatment for pruritus is by no means high.[11]

It is known that renal dysfunction and hemodialysis cause abnormal blood concentration of metals such as zinc that are involved in maintaining skin homeostasis.[12–15] Furthermore, several metals, such as iron and zinc, are found in blood cells as well as serum. Therefore, when examining blood met-

*To whom correspondence should be addressed. e-mail: knishida@suzuka-u.ac.jp; zooi@suzuka-u.ac.jp

al concentrations, its concentrations in blood cells and whole blood cannot be ignored. Few studies have reported simultaneous measurement of the concentrations of multiple metals in whole blood and serum. The purpose of this study is to examine the relationship between pruritus and metal concentrations in serum and whole blood in hemodialysis patients in order to develop new potential treatments.

MATERIALS AND METHODS

Study Participants Between November 2017 and March 2018, patients who underwent hemodialysis treatment at Shoujunkai Takeuchi Hospital (Tsu city, Mie prefecture, Japan) were enrolled in the study. Patients with wounds or obvious skin lesions on the forearm or the area to be observed, and patients taking zinc preparations or supplements were excluded from the study. The control group included non-dialysis patients without scratches.

Itch Assessment The presence or absence and the degree of itch were evaluated using Shiratori's criteria.[16,17] Shiratori's criteria are used to subjectively evaluate the symptoms of itch during the day and night and are rated on a 5-point scale ranging from 0 to 4 points. Subjective symptom scores of 0 and 1 do not include scratches. Thus, patients with a daytime or nighttime score of 2 or higher were classified as the scratching group, and patients with both daytime and nighttime scores of 1 or lower were classified as the non-scratching group.

Measurement of Blood Metal Concentrations Blood was collected at the start of hemodialysis in order to measure the concentration of metals (magnesium, calcium, manganese, iron, copper, zinc) in serum and the whole blood. Blood was collected between 9 am and 2 pm. BD Vacutainer® blood collection tubes were used to collect serum samples, and BD Vacutainer® heparin blood collection tubes were used for whole blood samples (3 mL each). Linkable anonymization of the samples was done by the information manager at Takeuchi Hospital. The blood samples were kept at 2–8°C and transported to Suzuka University of Medical Science within a few hours of collection. Serum was separated from the samples by centrifugation at 3000 rpm for 10 min using a centrifuge LC-200 (TOMY DIGITAL BIOLOGY CO., LTD., Tokyo, Japan). Subsequently, serum and whole blood samples were frozen at –20°C and transported to Kyoto Pharmaceutical University under the responsibility of a researcher at Suzuka University of Medical Science. The metal concentration in serum and whole blood was measured by an inductively coupled plasma-mass spectrometer (ICP-MS) at Kyoto Pharmaceutical University. Details of the ICP-MS method are described in the next section.

On the day of testing, both the patients and those in the control group took no food prior to blood collection and blood was collected at the same time interval.

Determination of Trace Metal Elements Concentration Determination of trace metal elements concentration was carried out by referring to previous studies.[18,19] Briefly, one hundred microliters of serum and whole blood samples were placed in tall 50 mL beakers and heated to 150°C on a hotplate. Then 2 mL of 60% (v/v) nitric acid (HNO_3 for poisonous metal determination; Kanto Chemical, Tokyo, Japan) was added. Three minutes after the addition of nitric acid, 2 mL of 60% (v/v) perchloric acid ($HClO_4$ for poisonous metal determination; Kishida Chemical, Osaka, Japan) was added. After additional 3 min, 2 mL of 30% (v/v) hydrogen peroxide (H_2O_2 for atomic absorption spectrochemical analysis; Kishida Chemical) was added. This process was repeated thrice, then another 2 mL of 30% (v/v) H_2O_2 was added and the samples were heated until digestion was complete. The liquid was evaporated, and the sample residues were cooled, then 5 mL of 5% (v/v) HNO_3 was added and the sample ash residues were dissolved overnight. The solutions were then transferred to sample cups. All tall beakers and sample cups used in this experiment were pretreated with 1% (v/v) HNO_3 to avoid metal contamination. The trace elements (magnesium, calcium, manganese, iron, copper, zinc) in the solutions were identified and quantitated by ICP-MS (Agilent7700/Mass Hunter, Agilent Technologies, Santa Clara, CA, USA). Standard curves were plotted by preparing 1,000 µg/mL (ppm) standard solutions of magnesium, calcium, manganese, iron, copper, and zinc (Fujifilm Wako Pure Chemical Industries Ltd., Osaka, Japan) and diluting them in 5% (v/v) HNO_3 to final metal concentrations of 0, 1, 2, 5, 10, 20, 50, 100, and 200 ng/mL (ppb). For quality control, 1 ng/mL (ppb) of a reference internal standard (indium; In) was measured along with the samples. The standard curves of each trace metal exhibited good linear regression (over r = 0.999) in the concentration range of 1–200 ng/mL (ppb).

Assessments of Skin Conditions A Tewameter® TM300 and Corneometer® CM825 (both Courage + Khazaka electronic GmbH, Koln, Germany) were used to measure transepidermal water loss (TEWL) and stratum corneum moisture content, respectively. TEWL is the amount of water that passively evaporates through skin to the external environment due to water vapor pressure gradient on both sides of the skin barrier and is expressed in $g/m^2 \cdot h$. Stratum corneum moisture content is expressed in arbitrary unit (a.u.).[20,21] Measurements were taken 3 times each within 15 min from the start of hemodialysis on the inner side of the forearm on the non-shunt limb side. The mean of the three measured values was used for statistical calculations. Patients were instructed not to use lotion or moisturizer on the forearm for one day prior to measurement, and to expose the forearm from their clothing for at least 15 min before measurement. The measurement conditions were an air temperature of 24.2 ± 1.3°C and a humidity of 40.0 ± 5.4%.

Statistical Analysis Data are expressed as mean ± S.D. The differences in stratum corneum moisture content and TEWL among the scratching group, non-scratching group, and control group were analyzed using the Tukey–Kramer multiple comparison test. The differences in serum and whole blood metal concentrations were analyzed using the Mann–Whitney U test, and the correlations between the stratum corneum moisture content or the TEWL and metal concentrations were analyzed using the Spearman correlation coefficient. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan, version 1.33), which is a graphical user interface for R (the R Foundation for Statistical Computing, Vienna, Austria, version 3.3.1). More precisely, it is a modified version of the R commander (version 2.3-0) designed to add statistical functions frequently used in biostatistics.[22] A p value < 0.05 was considered significant.

Ethical Approval The study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board of Suzuka University of Medical Science (Approval No.318, June 29, 2017).

Informed Consent Written informed consent was obtained from all patients.

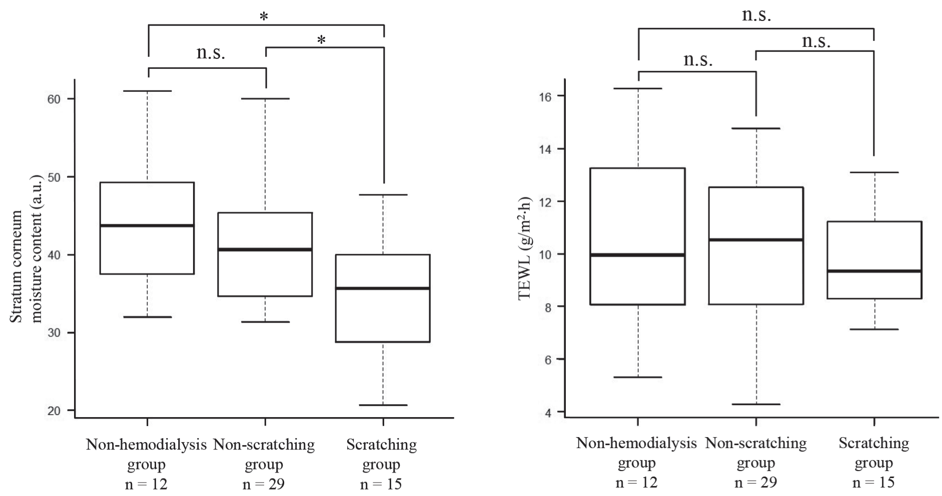

Fig. 1. Comparison of Stratum Corneum Moisture Content and TEWL between Scratching, Non-Scratching and Control Groups

The presence or absence and the degree of itch were evaluated using Shiratori's criteria. Patients with a daytime or nighttime score of 2 or higher were classified as the scratching group (n = 29), and patients with both daytime and nighttime scores of 1 or lower were classified as the non-scratching group (n = 15). Date show mean ± S.D.*$p < 0.05$, one way ANOVA with Tukey–Kramer multiple comparison test. There are no significant differences in TEWL.

RESULTS

The study group consisted of 44 patients (25 males, 19 females, age: 66.5 ± 10.9 years). The control group consisted of 12 patients (3 males, 9 females, age: 79.8 ± 5.9 years). Within the study group, the scratching and non-scratching groups consisted of 15 patients (8 males, 7 females, age: 66.5 ± 9.5 years) and 29 patients (17 males, 12 females, age: 70.7 ± 7.5 years), respectively.

The stratum corneum moisture content and TEWL were 34.9 ± 8.3 a.u. and 9.8 ± 1.9 $g/m^2 \cdot h$ in the scratching group, 41.5 ± 8.3 a.u. and 10.5 ± 2.7 $g/m^2 \cdot h$ in the non-scratching group, and 43.6 ± 8.4 a.u. and 10.5 ± 3.3 $g/m^2 \cdot h$ in the control group. The stratum corneum moisture content in the scratching group was significantly lower than that in the non-scratching and control groups, indicating the development of dry skin in the scratching group. On the other hand, no significant difference in TEWL was observed among the groups (Fig. 1). In the study group, there was a significant negative correlation between the stratum corneum moisture content and the sum of the daytime and nighttime Shiratori scores (Fig. 2).

Serum and whole blood metal concentrations (ng/mL) in the study and control groups are shown in Table 1. In the study group, serum magnesium and whole blood calcium, manganese (Mn), and zinc values were significantly higher than those in the control group, while serum calcium, iron, copper, and zinc, and whole blood iron were significantly lower than those in the control group. In particular, the level of whole blood Mn in the study group was remarkably high, approximately 8 times larger than that in the control group (168 ± 42 ng/mL vs 22 ± 15 ng/mL). In contrast, no significant difference was observed between the scratching and non-scratching groups.

We also examined the correlation between the stratum corneum moisture content, TEWL, and all metal concentrations in patients in the study group. There was a significant negative correlation between the stratum corneum moisture content and the whole blood Mn concentration (Fig. 3). No other significant correlation was observed.

DISCUSSION

It is known that blood metal concentrations in hemodialysis patients vary compared to those in healthy individuals. Our study demonstrated that the whole blood Mn levels are significantly elevated in hemodialysis patients. In a previous study of 40 adult hemodialysis patients (18 males, 22 females, 52.4 ± 11.6 years) in Japan, high blood cell Mn and low serum Mn were observed.[23] In another study that examined 57 patients in Spain, serum nickel, whole blood arsenic and lead were found to be high, while serum copper, zinc, and selenium levels were low, but serum and whole blood Mn levels were normal.[24] A study of 53 adult hemodialysis patients aged between 23 and 79 years in Iran reported high serum magnesium and zinc as measured by atomic absorption spectrometry.[25] On the other hand, a meta-analysis that examined blood metal concentrations in serum, plasma, and whole blood showed that dialysis patients have high copper and low Mn and zinc.[14] The inconsistencies between studies may be due to the low number

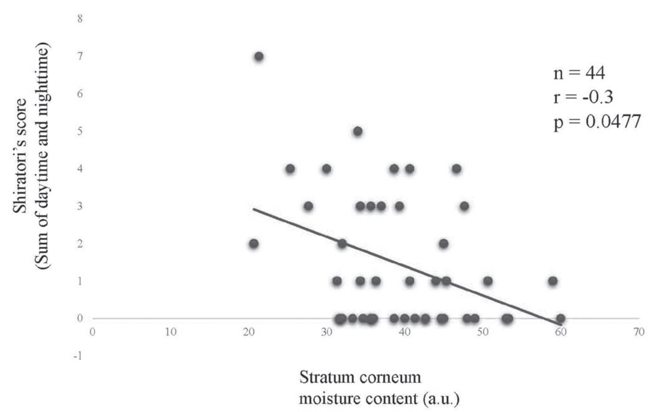

Fig. 2. Correlation between Stratum Corneum Moisture Content and Shiratori's Score

Evaluation with Spearman's correlation coefficient shows a significant negative correlation between the stratum corneum moisture content and Shiratori's score.

Table 1. Concentrations of Trace Elements in Hemodialysis and Non-Dialysis Group

		Magnesium	Calcium	Manganese	Iron	Copper	Zinc
Serum	Non-hemodialysis group	17200 ± 1800	**83400 ± 9700**	21 ± 10	**2680 ± 800**	**1330 ± 170**	**2430 ± 1310**
	Hemodialysis group	**19700 ± 3600**	74500 ± 9600	15 ± 11	1540 ± 590	1050 ± 290	670 ± 640
	Non-scratcing group	19900 ± 3600	73200 ± 9900	16 ± 12	1590 ± 630	1040 ± 300	610 ± 640
	Scratching group	19500 ± 3600	76900 ± 8600	14 ± 9	1440 ± 510	1070 ± 270	780 ± 620
	p value	0.0102	0.0109	n.s. (0.0737)	< 0.00001	0.00117	< 0.00001
Whole blood	Non-hemodialysis group	17500 ± 6700	32000 ± 12000	22 ± 15	**288000 ± 90000**	930 ± 290	4820 ± 2510
	Hemodialysis group	18700 ± 3700	**42000 ± 12200**	**168 ± 42**	228000 ± 45000	840 ± 440	**9260 ± 5130**
	Non-scratcing group	18700 ± 3700	40800 ± 12300	168 ± 44	219000 ± 47000	880 ± 520	8980 ± 5330
	Scratching group	18700 ± 3700	44400 ± 12000	168 ± 41	245000 ± 36000	770 ± 210	9790 ± 4870
	p value	n.s. (0.629)	0.0166	< 0.00001	0.0139	n.s. (0.0954)	0.0019

Unit: ng/mL
Data are expressed as mean ± S.D.
Hemodialysis group: n = 44, Non-hemodialysis group: n = 12.
Of hemodialysis group, Scratching group: n = 15, Non-scratching group: n = 29.
The boldface shows significantly larger.

of subjects in each study and the difference in measurement methods. Blood metal concentrations in hemodialysis patients were compared with the results from previous studies in Japanese patients, as in the present study. The results for serum zinc, iron, copper, and Mn described in the literature were in general agreement with the results of previous studies.[23,26,27) Blood metal concentrations in the control group were compared with those in healthy Japanese as reported by the Japan Association of Prefectural and Municipal Public Health Institutes,[28] and were in general agreement, except for serum zinc, whole blood magnesium, and iron. The control group in the present study was limited to the elderly, which may have led to differences from previous studies. Furthermore, the studies described above did not examine the association between blood metal levels and adverse events such as itching that affect patient QOL.

In our study, serum and whole blood concentrations of six metals were simultaneously measured by ICP-MS. Because ICP-MS can simultaneously measure multiple samples under the same conditions, it is expected to provide highly reliable results. Low serum concentrations were observed for iron, copper, zinc, and Mn and for whole blood iron in hemodialysis patients. This result was considered reasonable because renal anemia develops in patients with chronic renal failure due to decreased production and secretion of erythropoietin, suggesting a decrease in blood iron levels. On the other hand, high concentrations of serum magnesium and in whole blood calcium, zinc, and Mn were observed. In our hemodialysis patients, the serum concentration of zinc was actually 1/3 or less than the normal value and more than twice the normal value in whole blood. The majority of zinc in the blood is present in red blood cells. This suggests that transporters expressed in red blood cells may be involved in fluctuations in blood zinc concentrations in hemodialysis patients. Experiments in mice have shown that ZNT1, Zip8, and Zip10 are expressed in red blood cells.[29] The increase of zinc concentration in red blood cells observed in this study may be due to the expression of Zip8 and Zip10 that specifically affect zinc uptake.

In the present study, the whole blood Mn levels in hemodialysis patients was about 8 times higher than those in non-dialysis patients. In addition, there was a negative correlation between the whole blood Mn and the stratum corneum moisture content. Furthermore, since the stratum corneum moisture content was significantly different depending on the presence or absence of scratching. But unfortunately there was no direct correlation between blood metal concentration and itching.

In recent years, it has become clear that many transporters are involved in maintaining the homeostasis of Mn *in vivo*.[30,31] Zinc transporters ZNT10, Zip8, and Zip14 have been reported to be involved in the influx and efflux of Mn and zinc from cells.[32] Zip8, which is reportedly expressed in red blood cells, might increase the uptake of Mn into red blood cells, thereby increasing the Mn concentration in the whole blood in hemodialysis patients.[29]

In addition to the above, it is known that eccrine sweat glands atrophy in hemodialysis patients and the amount of sweating decreases.[33,34] Human sweat contains a certain amount of Mn and other metals.[35] In hemodialysis patients, metal excretion outside the body is decreased due to decreased sweating, which may affect the whole blood Mn levels.

Mn is widely contained in variety of foods, with vegetables and especially whole grains, beans, nuts, and tea leaves being the major source.[36] Since the normal nutritional requirements of Mn is very small, there is almost no deficiency in the normal diet. Excessive levels of Mn are limited to rare cas-

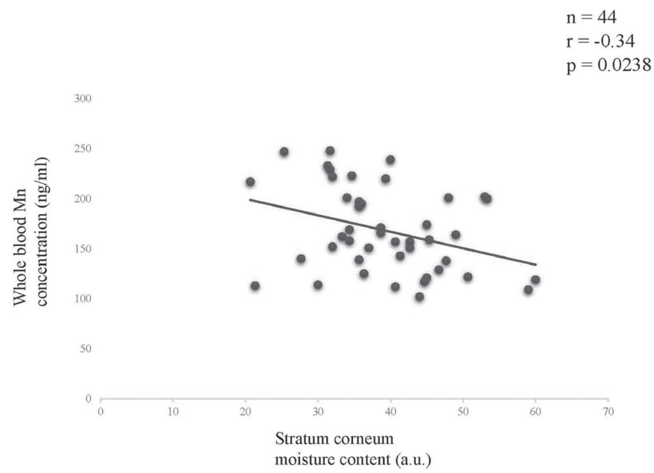

Fig. 3. Correlation between Stratum Corneum Moisture Content and Whole Blood Manganese Concentration

The correlation between the stratum corneum moisture content, TEWL, and all metal concentrations were tested for the patients in the study group. Examination with Spearman's correlation coefficient shows a significant negative correlation between stratum corneum moisture content and whole blood Mn concentration.

es such as parenteral nutrition, dust inhalation, and drinking contaminated water, and major Mn overload has been known to cause neurological symptoms.[37–39] On the other hand, there have been few reports of overexposure to skin. Mn functions as a component and an activator of metalloenzymes *in vivo*. Enzymes containing Mn include arginine-degrading enzyme, lactate decarboxylase, and Mn superoxide dismutase (MnSOD).[40,41] It has been reported that MnSOD, mainly localized in mitochondria, may regulate both immunosuppressive and proinflammatory signals through alteration of dendritic cells and T cell function in the skin and may be involved in the defense of inflammation due to aging.[42] Since blood Mn concentration does not correlate with tissue Mn concentration,[43] the high blood Mn concentration found in the present study does not indicate an excessive amount of Mn in the body. In hemodialysis patients, it was speculated that Mn in tissues was transferred to the blood by some other factors. As a result, the activity of MnSOD in mitochondria may be reduced, and this may be a factor in developing dry skin. In order to examine this hypothesis further, it is necessary to research Mn concentration in tissues as well as in whole blood.

The major limitation of the present study is that age is significantly different between the study group and the control group. However, the correlation between whole blood Mn and stratum corneum moisture content, which is an important result in the present study, was recognized even in the study group. Also, the small sample size limits the statistical power of the study. Furthermore, since this study is a cross-sectional study, causality cannot be proven.

Our study suggests that the high level of whole blood Mn in hemodialysis patients is involved in the development of dry skin. Dry skin is commonly associated with a decrease in skin barrier function, and is known to have adverse effects on daily life that include skin wounds, soft tissue infections, reduced ability to concentrate, and sleep disorders, in addition to itching.[44] Skin hydration is generally lower in hemodialysis patients. Itching in hemodialysis patients is caused by a variety of factors, including dry skin. Our study showed at least an association between whole blood manganese levels and dry skin.

Conclusions Whole blood Mn concentration in hemodialysis patients was 8 times higher than that in non-dialysis patients. Furthermore, a negative correlation was observed between whole blood Mn concentration and stratum corneum moisture content, and stratum corneum moisture content was significantly different depending on the presence or absence of scratching. Patients with high levels of whole blood Mn exhibited dry skin and developed itching. Further study of the role of elevated Mn may lead to the development of methods to improve dry skins in hemodialysis patients.

Acknowledgment We would like to thank the staff of the artificial kidney center of Takeuchi Hospital for their cooperation for the present study.

This work was supported in part by JSPS KAKENHI Grant Numbers JP18H05299 (K.O. and K.N.).

We thank Dr. M. Kato for critical reading.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Japanese Society for Dialysis Therapy. “An overview of regular dialysis treatment in japan as of DEC. 31, 2019.” https://docs.jsdt.or.jp/overview/file/2019/pdf/02.pdf, cited 4 July, 2021.
2) Mettang T, Pauli-Magnus C, Alscher DM. Uraemic pruritus--new perspectives and insights from recent trials. *Nephrol. Dial. Transplant.*, **17**, 1558–1563 (2002).
3) Merkus MP, Jager KJ, Dekker FW, de Haan RJ, Boeschoten EW, Krediet RT. Physical symptoms and quality of life in patients on chronic dialysis: results of The Netherlands Cooperative Study on Adequacy of Dialysis (NECOSAD). *Nephrol. Dial. Transplant.*, **14**, 1163–1170 (1999).
4) Kimata N, Fuller DS, Saito A, Akizawa Fukuhara TS, Pisoni RL, Robinson BM, Akiba T. Pruritus in hemodialysis patients: Results from the Japanese Dialysis Outcomes and Practice Patterns Study (JDOPPS). *Hemodial. Int.*, **18**, 657–667 (2014).
5) Pisoni RL, Wikstrom B, Elder SJ, Akizawa T, Asano Y, Keen ML, Saran R, Mendelssohn DC, Young EW, Port FK. Pruritus in haemodialysis patients: International results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrol. Dial. Transplant.*, **21**, 3495–3505 (2006).
6) Biró T, Ko MC, Bromm B, Wei ET, Bigliardi P, Siebenhaar F, Hashizume H, Misery L, Bergasa NV, Kamei C, Schouenborg J, Roostermann D, Szabó T, Maurer M, Bigliardi-Qi M, Meingassner JG, Hossen MA, Schmelz M, Steinhoff M. How best to fight that nasty itch - from new insights into the neuroimmunological, neuroendocrine, and neurophysiological bases of pruritus to novel therapeutic approaches. *Exp. Dermatol.*, **14**, 225–240 (2005).
7) Keithi-Reddy SR, Patel TV, Armstrong AW, Singh AK. Uremic pruritus. *Kidney Int.*, **72**, 373–377 (2007).
8) Ozen N, Cinar FI, Askin D, Mut D. Uremic pruritus and associated factors in hemodialysis patients: A multi-center study. *Kidney Res. Clin. Pract.*, **37**, 138–147 (2018).
9) Vila T, Gommer J, Scates AC. Role of gabapentin in the treatment of uremic pruritus. *Ann. Pharmacother.*, **42**, 1080–1084 (2008).
10) Rayner H, Baharani H, Smith S, Suresh V, Dasgupta I. Uraemic pruritus: relief of itching by gabapentin and pregabalin. *Nephron Clin. Pract.*, **122**, 75–79 (2012).
11) Rayner HC, Larkina M, Wang M, Graham-Brown M, van der Veer SN, Ecder T, Hasegawa T, Kleophas W, Bieber BA, Tentori F, Robinson BM, Pisoni RL. International Comparisons of Prevalence, Awareness, and Treatment of Pruritus in People on Hemodialysis. *Clin. J. Am. Soc. Nephrol.*, **12**, 2000–2007 (2017).
12) Prasad AS. Impact of the discovery of human zinc deficiency on health. *J. Am. Coll. Nutr.*, **28**, 257–265 (2009).
13) Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. *Adv. Nutr.*, **4**, 176–190 (2013).
14) Tonelli M, Wiebe N, Hemmelgarn B, Klarenbach S, Field C, Manns B, Thadhani R, Gill J. Alberta Kidney Disease Network, Trace elements in hemodialysis patients: a systematic review and meta-analysis. *BMC Med.*, **7**, 25 (2009).
15) Filler G, Felder S. Trace elements in dialysis. *Pediatr. Nephrol.*, **29**, 1329–1335 (2014).
16) Nishimoto Y, Nishimoto Y, Saito K, Sugisaki T. Reduction of pruritus by increasing dialyzer rinsing solution in hemodialysis patients. *J. Jpn. Soc. Dial. Ther.*, **33**, 287–295 (2000).
17) Tanaka H, Kishimoto T, Yamagami S, Nishio S, Iritani Y, Takemoto Y. Clinical effects of azelastine hydrochloride in hemodialysis patients with pruritus cutaneous. *J. Jpn. Soc. Dial. Ther.*, **28**, 389–395 (1995).
18) Nagatani Y, Imamura Y, Nakamura T, Yamashita K, Okuno M, Yasui H, Hiraoka J, Niigata R, Kono K, Hyogo Y, Suto H, Takenaka K, Funakoshi Y, Toyoda M, Kiyota N, Minami H, Pharmacokinetics of Oxaliplatin in a Hemodialysis Patient with Metastatic Colon Cancer. *Int J Oncol Res.*, 2 017 (2019).
19) Nakamura T, Takahashi M, Niigata R, Yamashita K, Kume M, Hirai M, Yasui H. Changes in blood concentrations of trace metals in cancer patients receiving cisplatin-based chemotherapy. *Biomed. Rep.*, **5**, 737–744 (2016).
20) Ooi k, Sasaki H, Yoshizawa N, Sugawa T, Kimura S, Ueda H, Numajiri S, Kojima S, Katayama T, Mizutani Y, Morimoto Y, Influence of

skin hydration on anesthetic effect of lidocaine tape in hemodialysis patients. *Jpn. J. Pharm. Health Care Sci.*, **34**, 1011–1016 (2008).
21) Takai Y, Hiramoto K, Nishimura Y, Uchida R, Nishida K, Ooi K. Association between itching and the serum zinc levels in patients with varicose veins. *J. Pharm. Health Care Sci.*, **3**, 1–6 (2017).
22) Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant.*, **48**, 452–458 (2013).
23) Hosokawa S. Manganese and nickel in chronic hemodialysis patients. *J. Jpn. Soc. Dial. Ther.*, **19**, 987–991 (1986).
24) Gómez de Oña C, Martínez-Morillo E, Gago González E, Vidau Argüelles P, Fernández Merayo C, Álvarez Menéndez FV. Variation of trace element concentrations in patients undergoing hemodialysis in the north of Spain. *Scand. J. Clin. Lab. Invest.*, **76**, 492–499 (2016).
25) Ahmadipour F, Mahjoub S, Pouramir M, Siahposht A, Afshar Naderi A, Absalan A. Determining Serum Zinc and Magnesium Levels in Hemodialysis Patients Could be Helpful for Clinicians. *Indian J. Clin. Biochem.*, **32**, 464–467 (2017).
26) Yamaya K, Tsuboi S, Saito H, Funyu T, Ooyama T, Goto S, Sera K. Trace element in hair and blood of hemodialysis patients. *NMCC ANNUAL REPORT.*, **20**, 109–114 (2013).
27) Hosokawa S, Nishitani H, Tomoyoshi T, Sawanishi K, Yoshida O. The relationship between trace elements and anemia in chronic hemodialysis patients. *J. Jpn. Soc. Dial. Ther.*, **20**, 323–327 (1987).
28) Hokkaido Institute of Public Health. "Concentrations of some metals in whole blood and plasma of normal adult subjects." <https://www.iph.pref.hokkaido.jp/Kankobutsu/Shoho/annual31/shoho310203.pdf>, cited 4 July, 2021.
29) Ryu MS, Lichten LA, Liuzzi JP, Cousins RJ. Zinc transporters ZnT1 (Slc30a1), Zip8 (Slc39a8), and Zip10 (Slc39a10) in mouse red blood cells are differentially regulated during erythroid development and by dietary zinc deficiency. *J. Nutr.*, **138**, 2076–2083 (2008).
30) Reddi AR, Jensen LT, Culotta VC. Manganese homeostasis in Saccharomyces cerevisiae. *Chem. Rev.*, **109**, 4722–4732 (2009).
31) Culotta VC, Yang M, Hall MD. Manganese transport and trafficking: lessons learned from Saccharomyces cerevisiae. *Eukaryot. Cell*, **4**, 1159–1165 (2005).
32) Jenkitkasemwong S, Wang CY, Mackenzie B, Knutson MD. Physiologic implications of metal-ion transport by ZIP14 and ZIP8. *Biometals*, **25**, 643–655 (2012).
33) Cawley EP, Hoch-Ligheti C, Bond GM. The eccrine sweat glands of patients in uremia. *Arch. Dermatol.*, **84**, 889–897 (1961).
34) Beerman H, Greenbaum CH. Renocutaneous syndromes. A review of some of the recent literature. *Am. J. Med. Sci.*, **243**, 517–532 (1962).
35) Cohn JR, Emmett EA. The excretion of trace metals in human sweat. *Ann. Clin. Lab. Sci.*, **8**, 270–275 (1978).
36) Ministry of Education. Culture, Sports, Science and Technology. "STANDARD TABLES OF FOOD COMPOSITION IN JAPAN - 2015 - (Seventh Revised Edition)." <https://www.mext.go.jp/a_menu/syokuhinseibun/1365297.htm>, cited 4 July 2021
37) Dobson AW, Erikson KM, Aschner M. Manganese neurotoxicity. *Ann. N. Y. Acad. Sci.*, **1012**, 115–128 (2004).
38) Peres TV, Schettinger MR, Chen P, Carvalho F, Avila DS, Bowman AB, Aschner M. Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies. *BMC Pharmacol. Toxicol.*, **17**, 57 (2016).
39) Erikson KM, Aschner M. Manganese: Its Role in Disease and Health. *Met. Ions Life Sci.*, 19 (2019).
40) Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. *Mol. Aspects Med.*, **26**, 353–362 (2005).
41) Wood RJ. Manganese and birth outcome. *Nutr. Rev.*, **67**, 416–420 (2009).
42) Scheurmann J, Treiber N, Weber C, Renkl AC, Frenzel D, Trenz-Buback F, Ruess A, Schulz G, Scharffetter-Kochanek K, Weiss JM. Mice with heterozygous deficiency of manganese superoxide dismutase (SOD2) have a skin immune system with features of "inflamm-aging". *Arch. Dermatol. Res.*, **306**, 143–155 (2014).
43) Matsuda A, Kimura M, Kataoka M, Ohkuma S, Sato M, Itokawa Y. Quantifying manganese in lymphocytes to assess manganese nutritional status. *Clin. Chem.*, **35**, 1939–1941 (1989).
44) Valdes-Rodriguez R, Stull C, Yosipovitch G. Chronic pruritus in the elderly: pathophysiology, diagnosis and management. *Drugs Aging*, **32**, 201–215 (2015).