Evaluating extremely low plasma ascorbate levels and reduction of plasma ascorbate levels by dialysis in Japanese hemodialysis patients

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Abbreviations: ANOVA, analysis of variance; AST, aspartate aminotransferase; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DHA, dehydroascorbic acid; EDTA, ethylenediaminetetraacetic acid; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; TIBC, iron-binding capacity; SEM, standard error of the mean; RDA, recommended dietary allowance
Abstract

Background: Low plasma ascorbate levels in hemodialysis patients have been reported worldwide; hence, many end-stage kidney disease patients are forced to restrict their diets, especially potassium-rich fruits and vegetables, to prevent hyperkalemia. In this study, we aimed to clarify whether plasma ascorbate levels are low in Japanese dialysis patients and whether plasma ascorbate levels fluctuate before and after dialysis. In addition, we aimed to clarify whether there are clinical test items that have a causal relationship with plasma ascorbate levels.

Methods: Plasma ascorbate levels in 27 chronic kidney disease (CKD) stage G3–G5 patients (mean age 84 years) and pre- and post-dialysis plasma ascorbate levels in 19 CKD stage G5D hemodialysis patients (mean age 79 years) were determined using high-performance liquid chromatography and electrochemical detection.

Results: Pre-dialysis plasma ascorbate levels in hemodialysis patients (12.0 ± 1.4 µM) were significantly lower (by 56%) than those in CKD stage G3–G5 patients (27.1 ± 2.7 µM). After dialysis, there was a 40% reduction in plasma ascorbate levels. Moreover, pre-dialysis ascorbate levels correlated significantly with plasma potassium levels.
Conclusions: The study results indicate that Japanese hemodialysis patients have lower plasma ascorbate levels than CKD stage G3–G5 patients and that these low plasma ascorbate levels in hemodialysis patients were further reduced by hemodialysis. To avoid the development of scurvy in hemodialysis patients, it is necessary to take sufficient ascorbate from supplements or medicines.

Keywords: ascorbate; chronic kidney disease (CKD); hemodialysis; hyperkalemia; oxalate; potassium; scurvy; vitamin C
Background

Vitamin C (L-ascorbic acid) is a water-soluble micronutrient and antioxidant that scavenges reactive oxygen species [1-3]. Under physiological pH conditions, ascorbic acid most commonly exists in its mono-anion form, ascorbate [4]. In addition to its antioxidant property, ascorbate contributes to numerous well-defined enzymatic reactions involving collagen hydroxylation, carnitine and norepinephrine biosynthesis, tyrosine metabolism, and peptide hormone amidation [5-7]. Many vertebrates have the ability to synthesize ascorbate from glucose de novo in the liver [8]. However, primates, including humans, are unable to synthesize ascorbate since they carry multiple mutations in the Gulo gene encoding L-gulono-γ-lactone oxidase, the last enzyme in the ascorbate biosynthesis pathway [9]. Therefore, humans must consume ascorbate from dietary sources such as fresh fruits and vegetables to prevent scurvy.

Scurvy is a condition that results from insufficient ascorbate in the body. Most scurvy symptoms such as anemia, weakness, and gingival bleeding are often seen in hemodialysis patients [10].

In the recent years, the increasing number of patients undergoing dialysis has become a social problem worldwide. Currently, there has been an increase in the mean age of incident dialysis
among patients aged ≥45 years, especially among those aged ≥65 years [11]. The proportion of patients aged ≥65 years at the end of 2012 was 65.5% in Japan, indicating the increase in dialysis incidence in the aging population [11].

Low plasma ascorbate levels have been observed in some hemodialysis patients for many years globally [12-18]. However, it is unclear why plasma ascorbate levels are low in hemodialysis patients. Hemodialysis patients are forced to have dietary restrictions, such as protein, salt, and potassium restriction. The consumption of fruits and vegetables that contain high amounts of ascorbate is also restricted due to the high potassium content.

In this study, we examined whether Japanese hemodialysis patients have low plasma ascorbate levels compared to non-hemodialysis-dependent patients with chronic kidney disease (CKD). In addition, we also examined whether dialysis reduces plasma ascorbate levels. Furthermore, we analyzed whether there are any clinical test items that have a causal relationship with plasma ascorbate levels in Japanese hemodialysis patients.
Methods

Ethical consideration

This study was conducted according to the principles expressed in the Declaration of Helsinki.

This study was approved by the Clinical Research Ethics Committee of the Tokyo Metropolitan Geriatric Medical Center, Tokyo, Japan (permit number: R18-40) and Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan (permit number: H30-25). All patients gave their written informed consent and patient anonymity is preserved.

Study patients

Patients with CKD stage G3–G5 (mean eGFR 23 mL/min/1.73 m²) (n=34) and CKD stage G5D hemodialysis (n=19) who had been regularly visiting an outpatient clinic of Tokyo Metropolitan Geriatric Medical Center from October 2018 through December 2019 were recruited in this study. A diagnosis of CKD was made based on the guidelines of the National Kidney Foundation Kidney Disease Outcomes Quality Initiative [19].

Hemodialysis regimen
Of the 19 patients, 10 were under maintenance hemodialysis and 9 patients were undergoing hemodiafiltration. The procedures were performed three times weekly for 9–12 h per week at a blood flow rate of 150–200 mL/min and dialysis flow rate of 500 mL/min using dialyzers with a surface area of 1.1–2.1 m². The dialysate sodium concentration was 140 mEq/L and the potassium concentration was 2.0 mEq/L. The mean duration of dialysis therapy was 3.6 years. The mean Kt/V was 1.3.

**Collection of blood and urine samples**

Blood samples for the measurement of clinical test items and ascorbate were obtained at the same time. For the determination of ascorbate levels, blood samples were drawn into a VENOJECT® collection tube (Terumo Corporation, Tokyo, Japan) containing ethylenediaminetetraacetic acid (EDTA)-2Na as an anticoagulant. All of the following procedures were performed within 2 h after sampling since we confirmed in advance that the values of ascorbate are unstable if it is beyond 2 h [20]. Plasma was obtained by centrifugation at 1,700 g for 10 min. After the plasma was collected, 0.5 mL of supernatant was immediately mixed with 0.5 mL of cold 10% metaphosphoric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 1 mmol/L of EDTA (Dojindo Laboratories, Kumamoto, Japan) and
centrifuged at 21,000 g for 15 min at 4 °C for the analysis of ascorbate level. After collecting the urine samples, 0.5 mL of urine was immediately mixed with 0.5 mL of cold 10% metaphosphoric acid containing 1 mmol/L of EDTA and centrifuged at 21,000 g for 15 min at 4 °C for the analysis of ascorbate level. All samples were stored at -80 °C until use.

Collection of blood from dialysis

To establish the basal plasma ascorbate levels in hemodialysis patients and the effect of hemodialysis, blood samples were drawn (from the arteriovenous fistula or catheter) prior to the start of dialysis (pre-dialysis sample) and immediately after ending the dialyzing period (post-dialysis sample).

Determination of ascorbate and dehydroascorbic acid (DHA)

Ascorbate and DHA, which is an oxidized form of L-ascorbic acid, levels were measured using high-performance liquid chromatography and electrochemical detection according to the methods described previously [21]. After thawing, the plasma and urine were centrifuged at 21,000 g for 10 min at 4°C. For determination of total ascorbate including DHA, the centrifugal supernatants were reduced with tris(2-carboxyethyl)phosphine hydrochloride for 2 h on ice.
After reduction, the reaction mixture was diluted with 5% metaphosphoric acid containing 0.5 mmol/L EDTA and analyzed for total ascorbate by high-performance liquid chromatography coupled with electrochemical detection. Separation was achieved on an Atlantis dC18 5-µm column (4.6 × 150 mm) combined with an Atlantis dC18 5-µm guard column (4.6 × 20 mm) from Nihon Waters (Tokyo, Japan). The mobile phase consisted of 50 mM phosphate buffer (pH 2.8), 540 µM EDTA, and 2% methanol at a flow rate of 1.3 mL/min, and electrical signals were recorded using an electrochemical detector with a glassy carbon electrode at +0.6 V. All electrical signal data from the electrochemical detector were collected using Waters Empower 2 software (Nihon Waters). The value of DHA was determined by subtracting ascorbate from total ascorbate. The ascorbate level of urine is immediately affected after a meal. Therefore, we evaluated the plasma ascorbate level in the samples whose urinary ascorbate was <0.5 mM.

**Study items**

The following data were collected from the medical records: age, gender, and clinical investigations, i.e., white blood cell, hemoglobin, hematocrit, platelet, total protein, albumin, C-reactive protein, aspartate aminotransferase (AST), blood urea nitrogen, creatinine, uric acid, sodium, potassium, calcium, phosphorus, triglyceride, total cholesterol, low-density lipoprotein
(LDL) cholesterol, iron, transferrin and iron-binding capacity (TIBC), ferritin, β2-microglobulin, prealbumin, hemoglobin A1c (HbA1c), and parathyroid hormone.

Statistical analysis

The results and clinical characteristic data are expressed as means ± standard error of the mean (SEM). The probability of statistical differences between experimental groups was determined by Welch’s t-test, paired t-test, and one-way analysis of variance (ANOVA) followed by Tukey-Kramer test. We verified that Pearson correlation coefficient between ascorbate concentration and clinical characteristics data is different from zero. Statistical differences were considered significant at $p < 0.05$. 
Results

Clinical characteristics of CKD stage G3–G5 and hemodialysis patients

A total of 34 CKD stage G3–G5 and 19 CKD stage G5D hemodialysis patients were enrolled.

Since seven CKD stage G3–G5 patients showed that urine ascorbate level was higher than 0.5 mM, suggesting supplemental intake of ascorbate just before collecting blood and urine, they were excluded from analysis. Therefore, the results of 27 CKD stage G3–G5 patients were used for analysis. Clinical characteristics of CKD stage G3–G5 and hemodialysis patients are shown in Table 1. The levels of the following were beyond the normal range and differed between CKD stage G3–G5 and hemodialysis patients: albumin, blood urea nitrogen, creatinine, phosphorus, and β2-microglobulin. Albumin values of both CKD stage G3–G5 and hemodialysis patients were below the normal range and blood urea nitrogen, creatinine, and β2-microglobulin values of both CKD stage G3–G5 and hemodialysis patients were higher than the normal range. Phosphorus values of hemodialysis-only patients were higher than the normal range.
Plasma ascorbate levels in CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in hemodialysis patients

The plasma ascorbate levels in 27 CKD stage G3–G5 and pre- and post-dialysis plasma ascorbate levels in 19 hemodialysis patients were measured (see Fig. 1). Pre-dialysis plasma ascorbate levels in hemodialysis patients (12.0 ± 1.4 µM) were significantly lower (by 56%) than that in CKD stage G3–G5 patients (27.1 ± 2.7 µM) (Fig. 1a). Moreover, after dialysis, there was a 40% reduction in the plasma ascorbate levels (7.2 ± 0.9 µM) (Fig. 1b). In addition, the individual pre- and post-dialysis plasma ascorbate levels and distribution of ascorbate levels in CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in hemodialysis patients are shown in Figure 1b and c.

Relationships between clinical characteristics and plasma ascorbate levels

We then analyzed the relationships between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients (Fig. 2). In Pearson correlation coefficient, pre-dialysis ascorbate levels correlated significantly with those of plasma potassium levels (positive correlation; Pearson correlation coefficients (r) = 0.6; p = 0.006) (Table 2). However, no
association was found between plasma ascorbate levels and other clinical characteristics except for plasma potassium levels.
In this study, we revealed that Japanese hemodialysis patients have low plasma ascorbate levels compared to non-hemodialysis CKD patients and these low plasma ascorbate levels in hemodialysis patients were further reduced by a single hemodialysis treatment session. Moreover, we found that ascorbate levels in hemodialysis patients correlated with those of plasma potassium levels. In general, hemodialysis patients are forced to restrict their diets, especially potassium-rich fruits and vegetables, to prevent hyperkalemia, which is a risk factor for dialysis morbidity and mortality [22]. Most of these fruits and vegetables also contain high amounts of ascorbate. Thereby, plasma ascorbate levels in Japanese hemodialysis patients might be correlated with plasma potassium levels.

The recommended dietary allowance (RDA) of vitamin C for a healthy adult is 100 mg per day in Japan and 90 mg and 75 mg per day for men and women, respectively, in the United States to prevent scurvy [23]. The average concentration of ascorbate in the plasma of healthy humans is 40–60 µM [24, 25]. When the plasma ascorbate concentration drops to below 11 µM, there is a risk of developing scurvy, which is thus conventionally considered deficient [24, 25]. In our previous report regarding chronic obstructive pulmonary disease (COPD) and plasma
ascorbate levels, we reported that plasma ascorbate levels were significantly lower in COPD patients (mean age 72.7 ± 6.9 years) than those in healthy elderly people (mean age 68.8 ± 3.8 years) using the same procedure and method as here [20]. Plasma ascorbate levels in COPD patients and healthy elderly people were 31.2 ± 2.2 µM and 42.3 ± 2.9 µM, respectively. Furthermore, the observed plasma levels in non-hemodialysis CKD patients and hemodialysis patients in the present study were lower than those in COPD patients. Plasma ascorbate levels in non-hemodialysis CKD patients and hemodialysis patients were 27.1 ± 2.7 µM and 12.0 ± 1.4 µM, respectively. Since there is a risk of developing scurvy when the plasma ascorbate concentration drops to below 11 µM [24, 25], many Japanese hemodialysis patients are likely to develop scurvy (see Fig. 3). Worldwide, many hemodialysis patients have developed scurvy [10, 26, 27].

Moreover, we tried to compare the percentages of DHA per total ascorbate in plasma from healthy elderly people, COPD patients, non-hemodialysis CKD patients, and hemodialysis patients, and found that the percentages of DHA in non-hemodialysis CKD patients (33.5%) and hemodialysis patients (37.4%) were notably higher percentages than those in COPD patients (12.4%) and healthy elderly people (10.0%) (see Fig. 3) [20]. High percentage of DHA
in non-hemodialysis CKD patients and hemodialysis patients may reflect a higher oxidative stress levels in their body.

Wang et al. [16] reported that plasma ascorbate concentrations were reduced by a median of 33% following dialysis. Deicher et al. [28] have also reported that hemodialysis causes a 50–75% decrease in plasma ascorbate levels. In the present study, plasma ascorbate levels reduced to 40% by hemodialysis. Thus, hemodialysis certainly reduces plasma ascorbate concentration in hemodialysis patients.

For a long time, there has been concern about the accumulation and deposition of oxalate with increased intake of vitamin C because oxalate is a breakdown product of vitamin C and is heavily excreted by the kidneys [29]. Oxalate crystallization occurs at levels above 30 mM [30] and high plasma oxalate levels were seen in hemodialysis patients [31-33]. However, in a recent prospective case series exploring high-dose intravenous vitamin C (15–100 g) administration, increased vitamin C intake was not associated with any cases of symptomatic renal stones and kidney injury [34]. Moreover, significant side effects of vitamin C are not reported in any of the mentioned controlled trials, including the most recent VITAMIN randomized trial [35]. CKD patients with higher levels of plasma calcium, phosphate, and parathyroid hormone have a high risk of death because CKD often causes abnormal calcium and phosphate metabolism...
and hyperparathyroidism [36-40]. Therefore, it is important to control the plasma calcium, phosphate, and parathyroid hormone levels in the non-hemodialysis CKD and hemodialysis patients [41]. Through a systematic review and meta-analysis, Ke et al. [42] have reported that vitamin C supplementation in CKD patients has no positive effect that influence the plasma phosphate or parathyroid hormone levels, but it increase plasma calcium levels in the short term.

In the present study, we could not detect any correlation between plasma ascorbate and plasma calcium, phosphate, and parathyroid hormone levels in Japanese hemodialysis patients. Meanwhile, we only found the positive correlation between plasma ascorbate and plasma potassium levels in Japanese hemodialysis patients. Perhaps Japanese hemodialysis patients that have dietary potassium restrictions to prevent hyperkalemia may limit their consumption of fresh vegetables and fruits that are rich in ascorbate. Thereby, there is a possibility that Japanese hemodialysis patients have low plasma ascorbate levels.

Recently, the increase of frailty in the elderly has become a social problem globally. The Dialysis Morbidity and Mortality Wave 2 cohort study revealed that >60% of end-stage kidney disease patients over the age of 40 met a definition of frailty, which impairs the prognosis [43]. Ascorbate is known to be one of the anti-aging factors because of its strong antioxidant
Therefore, ascorbate may be causally associated with life prognosis and aging in hemodialysis patients.

Conclusion

Japanese hemodialysis patients have low plasma ascorbate levels and are likely to develop scurvy. Furthermore, their plasma ascorbate levels are reduced by approximately 40% by a single hemodialysis. The cause of the low plasma ascorbate levels in hemodialysis patients may be due to the decreased intake of ascorbate from fresh fruits and vegetables due to the strict restriction of potassium intake. To avoid the development of scurvy in hemodialysis patients, it is necessary to consume sufficient ascorbate from supplements or medicine because of the body’s inability to synthesize ascorbate.
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Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

MI, TT, WY, NM, and AI designed the research; MI, TT, YT, AS, TY, and AI conducted the experiments; YD, MI, TT, WY, and AI analyzed the data; and YD, MI, TT, YT, AS, TY, WY, NM, and AI wrote the manuscript and had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

The authors declare no conflicts of interest.
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Figure Legends

Fig. 1 Plasma ascorbate levels in non-hemodialysis CKD stage G3–G5 patients and pre- and post-dialysis plasma ascorbate levels in CKD stage G5D hemodialysis patients. Total ascorbate levels were determined as described in Methods. (a, b) Dots in boxplots are expressed ascorbate levels in CKD stage G3–G5 (n=27) and hemodialysis (n=19) patients. Center lines are expressed median value of each groups. (c) Distributions of ascorbate levels in each group. P < 0.05 by (a) Welch’s t-test and (b) paired t-test.

Fig. 2 Scatterplots between clinical characteristics and pre-dialysis plasma ascorbate levels in hemodialysis patients. Dots are expressed data of individual hemodialysis patients (n=19). Blue and red lines are regression lines of data sets between ascorbate levels and clinical data. Pearson correlation coefficients are described in Table 2.

Fig. 3 Plasma ascorbate levels in healthy controls (n=28) [20] and chronic obstructive pulmonary disease (COPD) (n=39) [20], non-hemodialysis chronic kidney disease (CKD) stage G3–G5 (n=27), and hemodialysis (n=19) patients. Ascorbate (blue column) and DHA (yellow
levels were determined as described in Methods. The average concentration of ascorbate in the plasma of healthy humans is 40–60 µM (blue zone). There is a risk of developing scurvy when the plasma ascorbate concentration drops to below 11 µM (red zone). Values are expressed as a mean ± SEM. $P < 0.05$ by one-way ANOVA followed Tukey-Kramer test.
Table 1 Clinical characteristics of CKD stage G3–G5 and hemodialysis patients

| Characteristic                | Normal range | CKD stage G3–G5 (n=27) | Hemodialysis (n=19) |
|-------------------------------|--------------|------------------------|---------------------|
| Age (years)                  | 83.9 ± 1.3   | 78.9 ± 2.5             |
| Sex (male/female)            | 10/17        | 9/10                   |
| White blood cell (x10^3/µl)   | M: 3.9–9.8   | M: 6.7 ± 0.6           | M: 6.5 ± 0.4        |
|                              | F: 3.5–9.1   | F: 7.0 ± 0.4           | F: 7.1 ± 1.1        |
| Hemoglobin (g/dL)            | M: 13.5–17.6 | M: 12.1 ± 0.4          | M: 11.3 ± 0.2       |
|                              | F: 11.3–15.2 | F: 11.8 ± 0.3          | F: 11.2 ± 0.2       |
| Hematocrit (%)               | M: 39.8–51.8 | M: 36.5 ± 1.2          | M: 34.3 ± 0.8       |
|                              | F: 33.4–44.9 | F: 36.2 ± 0.7          | F: 35.1 ± 0.8       |
| Platelet (x10^4/µl)          | M: 13.1–36.2 * | M: 20.5 ± 2.0          | M: 23.8 ± 2.3       |
|                              | F: 13.0–36.9 | F: 22.1 ± 1.4          | F: 16.0 ± 1.9       |
| Total protein (g/dL)         | 6.7–8.3 *    | 6.9 ± 0.1              | 6.3 ± 0.1           |
| Albumin (g/dL)               | 3.8–5.2 *    | 3.6 ± 0.1              | 3.1 ± 0.1           |
| C-Reactive protein (mg/dL)   | < 0.3        | 0.4 ± 0.1              | 0.8 ± 0.6           |
| AST (IU/L)                   | 37.0–125.0   | 21.2 ± 1.0             | 24.7 ± 8.4          |
| Blood urea nitrogen (mg/dL)  | 8.0–22.0 *   | 32.9 ± 2.8             | 62.7 ± 3.1          |
| Creatinine (mg/dL)           | M: 0.6–1.0 * | M: 2.0 ± 0.3           | M: 10.4 ± 0.6       |
|                              | F: 0.5–0.8 * | F: 2.1 ± 0.3           | F: 7.4 ± 0.4        |
| Uric acid (mg/dL)            | M: 3.7–7.0   | M: 6.0 ± 0.3           | M: 5.9 ± 0.5        |
|                              | F: 2.5–7.0   | F: 6.3 ± 0.4           | F: 5.9 ± 0.5        |
| Sodium (mEq/L)               | 136.0–147.0 * | 140.4 ± 0.7           | 137.7 ± 0.8         |
| Potassium (mEq/L)            | 3.6–5.0 *    | 4.4 ± 0.1              | 4.9 ± 0.2           |
| Calcium (mg/dL)              | 8.5–10.2 *   | 9.1 ± 0.1              | 8.5 ± 0.1           |
| Phosphorus (mg/dL)           | 2.4–4.3 *    | 3.8 ± 0.1              | 5.1 ± 0.3           |
| Triglyceride (mg/dL)         | 50.0–149.0 * | 150.9 ± 14.1          | 108.7 ± 7.5         |
| Total cholesterol (mg/dL)    | 150.0–219.0 *| 200.9 ± 11.3          | 152.8 ± 8.7         |
| LDL cholesterol (mg/dL)      | 70.0–139.0 * | 109.5 ± 6.8           | 81.2 ± 6.1          |
| Iron (µg/dL)                 | M: 54.0–200.0 * | M: 66.2 ± 4.4         | M: 62.8 ± 14.9      |
|                              | F: 48.0–154.0 | F: 76.6 ± 6.8         | F: 47.5 ± 15.5      |
| TIBC (µg/dL)                 | M: 253.0–365.0 | M: 234.6 ± 5.2       | M: 251.7 ± 12.8     |
|                              | F: 246.0–410.0 | F: 253.6 ± 8.6       | F: 230.9 ± 15.5     |
| Ferritin (ng/dL)             | M: 3940–34,000 | M: 159.6 ± 27.3      | M: 105.9 ± 27.9     |
| Parameter                      | Value          |
|-------------------------------|----------------|
| β2-Microglobulin (mg/L)       | 1.0–1.9 *      |
| Prealbumin (mg/dL)            | 22.0–40.0      |
| HbA1c (%)                     | 4.6–6.2        |

Values are presented as the mean ± SEM. * significant difference at \( p < 0.05 \)

M, male; F, female; CKD, chronic kidney disease; AST, aspartate aminotransferase; LDL, low-density lipoprotein; TIBC, total iron-binding capacity; HbA1, hemoglobin A1c
| Characteristic                  | r      | p-value |
|--------------------------------|--------|---------|
| Age                            | 0.44   | 0.056   |
| Dry weight                     | 0.02   | 0.934   |
| White blood cell               | 0.12   | 0.617   |
| Hemoglobin                     | 0.18   | 0.461   |
| Hematocrit                     | 0.22   | 0.362   |
| Platelet                       | -0.38  | 0.104   |
| Total protein                  | 0.02   | 0.944   |
| Albumin                        | -0.12  | 0.632   |
| C-Reactive protein             | 0.40   | 0.087   |
| AST                            | 0.30   | 0.206   |
| Blood urea nitrogen            | 0.05   | 0.835   |
| Creatinine                     | -0.12  | 0.633   |
| Uric acid                      | -0.05  | 0.828   |
| Sodium                         | 0.15   | 0.537   |
| Potassium *                    | 0.60   | 0.006   |
| Calcium                        | 0.05   | 0.829   |
| Phosphorus                     | -0.14  | 0.578   |
| Triglyceride                   | 0.30   | 0.206   |
| Total cholesterol              | 0.32   | 0.179   |
| LDL cholesterol                | 0.18   | 0.454   |
| Iron                           | 0.01   | 0.962   |
| TIBC                           | -0.33  | 0.161   |
| Ferritin                       | 0.44   | 0.060   |
| β2-Microglobulin               | 0.18   | 0.465   |
| Prealbumin                     | 0.00   | 0.995   |
| HbA1c                          | -0.19  | 0.430   |
| Parathyroid hormone            | -0.06  | 0.816   |

* p-value < 0.05

AST, aspartate aminotransferase; LDL, low-density lipoprotein; TIBC, total iron-binding capacity; HbA1, hemoglobin A1c
Figure 1
Figure 2
Figure 2
Figure 3