Research Article

Hypercalcemia Is a Risk Factor for the Progression of Aortic Calcification in Kidney Transplant Recipients

Toshihide Naganuma^a Yoshiaki Takemoto^a Junji Uchida^a
Tatsuya Nakatani^a Daijiro Kabata^b Ayumi Shintani^b

^aDepartment of Urology, Osaka City University Graduate School of Medicine, Osaka, Japan;

^bDepartment of Medical Statistics, Osaka City University Graduate School of Medicine, Osaka, Japan

Keywords

Agatston score · Vascular calcification · Kidney transplantation · Multi-slice computed tomography · Risk factor · Infrarenal abdominal aorta

Abstract

Background/Aims: Vascular calcification is common and progressive in chronic kidney disease, including kidney transplant recipients (KTRs). However, the risk factors associated with the progression of aortic calcification (AoC) in KTRs have not been fully elucidated. In the present study, we evaluated AoC and examined the factors associated with its advancement in KTRs. **Materials:** This was a prospective longitudinal study that included 98 KTRs. We quantitatively investigated infrarenal abdominal AoC using the Agatston score, as measured by multi-slice computed tomography. After the baseline investigation, a follow-up scan was performed after 3 years, and the Agatston scores were obtained again. The changes in laboratory data affecting the 2nd Agatston scores were examined by multivariable analysis using non-linear regression after adjustment for several confounders. **Results:** The 2nd Agatston scores were significantly greater than the baseline Agatston scores ($p < 0.001$). After adjustment for the confounders, the change in corrected serum calcium exhibited a significant non-linear correlation with the 2nd Agatston scores ($p = 0.022$ for non-linearity/ $p = 0.031$ for the effect of corrected serum calcium). Moreover, an interaction was present from the baseline AoC in the effect of corrected serum calcium on the progression of AoC, and the effect of hypercalcemia was greater in patients with higher baseline Agatston scores ($p = 0.049$). **Conclusion:** The present study revealed that hypercalcemia is a risk factor for the development of infrarenal abdominal AoC in KTRs. Furthermore, the effect of hypercalcemia was greater in patients with more severe vascular calcification.

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Toshihide Naganuma, MD, PhD
Department of Urology
Osaka City University Medical School
1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585 (Japan)
E-Mail spxd48k9@aria.ocn.ne.jp

Introduction

Even after successful kidney transplantation, cardiovascular disease (CVD) remains the leading cause of death among kidney transplant recipients (KTRs); with a 3.5–5% annual risk of fatal or nonfatal CVD events compared with the general population, even after adjustment for risk factors [1, 2]. In the general population, it is well established that vascular calcification (VC) is a prognostic marker of CVD events, and cardiovascular mortality [3–6]. Moreover, VC is highly prevalent and often progressive in patients with chronic kidney disease (CKD) [7, 8], particularly in dialysis patients [9, 10], and the presence and progression of VC is closely associated with the increased morbidity and mortality of CVD [7–10]. There have been many reports of advanced VC in kidney transplant recipients (KTRs) [11–23], and several studies have shown that VC strongly predicts the morbidity and mortality of CVD in KTRs [12, 18, 20]. However, most of these findings were based upon observational studies in which coronary artery calcification (CAC) was evaluated quantitatively using the Agatston score [11, 13–23], whereas only a few studies have assessed aortic calcification (AoC) [12, 14, 16, 17]. In this study, we performed a quantitative assessment of infrarenal abdominal AoC, using the Agatston score by multi-slice computed tomography (CT) and investigated the risk factors for the progression of VC in KTRs. In addition, the Agatston scores exhibiting greatly distorted data distribution were analyzed using non-linear regression analysis in consideration of any non-linear association.

Materials and Methods

Study Design and Participants

This was a prospective longitudinal study that included 98 KTRs. The inclusion criteria were (1) Consecutive, adult KTRs, (2) Out-patients of Osaka City University Hospital, (3) Patients who were more than 6 months post-transplant, and (4) Patients who gave informed consent prior to their participation in the study. The exclusion criteria were (1) Patients with malignancy, clinically overt infection, acute or severe illness, and (2) Patients who were pregnant or possibly pregnant. We planned to recruit participants from April 2010 to March 2014. Of the 111 patients that underwent baseline multi-slice CT, 4 cases with incomplete records were excluded, 9 cases were lost to follow-up: 6 cases died, 2 cases initiated dialysis and one transferred to another hospital, resulting in 98 cases that underwent a 2nd CT and were examined for this study. This study protocol was conducted in accordance with the Principles of the Declaration of Helsinki and the Declaration of Istanbul, and was approved by the ethics committee of the Osaka City University Graduate School of Medicine (No. 1415).

The demographic and baseline clinical data are presented in Table 1.

Measurement of the Agatston Score by Multi-Slice Computed Tomography

The protocol for multi-slice CT for measurement of Agatston score was derived from our previous study [24] and the protocol is provided in detail in the online supplementary material (see www.karger.com/doi/10.1159/000501740). We quantitatively investigated infrarenal abdominal AoC using the Agatston score, as measured by multi-slice CT from the renal artery to the bifurcation [24]. After the baseline investigation, a second multi-slice CT was performed after 3 years, and the Agatston scores were determined again.

The presence of AoC was defined by an Agatston score of >0. The progression of calcification was defined as the difference between the follow-up square-root transformed score (SRC) and the baseline SRC ≥ 2.5 [25]. A calcium score >30 has been reported to be highly reproducible (8–10% interscan variability) [26]. Therefore, we calculated the annualized

Table 1. Baseline characteristics of the study subjects (n = 98)

Variables	Median (IQR) or percentage
Age, years	49 (40, 58)
Sex (male), %	62.2
Body mass index	20.7 (18.9, 22.5)
Dialysis duration, years	3.4 (1.1, 9.9)
Time from transplantation, years	5.8 (2.8, 10.0)
Living donor, %	79.4
Calcineurin inhibitor (cyclosporine), %	61.2
eGFR, mL/min/1.73 m ²	48.5 (39.9, 54.4)
Serum creatinine, mg/dL	1.2 (1.0, 1.5)
Diabetes mellitus, %	23.5
HbA1c, %	6.0 (5.6, 6.4)
Hypertension, %	88.8
Systolic blood pressure, mm Hg	120 (113.3, 130)
Diastolic blood pressure, mm Hg	74 (67.3, 78.8)
Dyslipidemia, %	33.7
Total cholesterol, mg/dL	197 (170, 221)
Triglycerides, mg/dL	104 (75, 132)
High density lipoprotein cholesterol, mg/dL	65 (50, 77)
Low density lipoprotein cholesterol, mg/dL	105.8 (87.2, 130.6)
Serum albumin, g/dL	4.1 (3.9, 4.3)
C-reactive protein, mg/dL	0.03 (0.01, 0.05)
Corrected calcium, mg/dL	10.0 (9.6, 10.3)
Phosphate, mg/dL	3.0 (2.6, 3.3)
i-PTH, pg/mL	78 (61, 115)
Smoking habit, %	6.2
Use of statins, %	35.1
Aortic calcification (Agatston score >0)	78.6

Values are expressed as the percentage for categorical variables and median (IQR) for continuous variables. IQR, interquartile range; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; i-PTH, intact parathyroid hormone.

percentage rate of progression only in those with a baseline score >30 [$100 \times (\text{second Agatston score} - \text{baseline Agatston score}) / (\text{baseline Agatston score})$ interval between scans].

Definition of Traditional Risk Factors

Subjects were admitted to our clinical research unit at 8:30~9:30 a.m. without taking immunosuppressive medications after an 8–12-h overnight fasting period. Fasting blood samples were drawn to determine the clinical data. Serum calcium was corrected by serum albumin, according to the formula: Corrected Ca [mg/dL] = measured Ca [mg/dL] + (4 – serum albumin [g/dL]), when serum albumin was below 4.0 g/dL.

Hypertension (HT) was defined by (a) the administration of antihypertensive agents and/or a history of this disorder; (b) a systolic blood pressure greater than 140 mm Hg; or (c) a diastolic blood pressure greater than 90 mm Hg; blood pressure was measured prior to the outpatient consultation. Diabetes mellitus was defined as (1) administration of insulin or oral antidiabetic agents, (2) prior diagnosis according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus of the American Diabetes Association [27]. Body mass index (BMI) was calculated by the equation [(weight in kilograms) / (square of height in meters)].

Statistical Analysis

To express the patients' baseline demographical and clinical characteristics, median and interquartiles were used for continuous variables whereas percentage was used for categorical variables. Differences between the baseline and 2nd Agatston scores were examined using the Wilcoxon rank sum test.

The effects of the candidates of risk factors on the 2nd Agatston scores were assessed with multivariable linear regression models. Each risk factor was included separately in a multivariable regression model adjusting for the baseline Agatston score, age, sex, donor source, eGFR, body mass index (BMI), days after transplantation, dialysis duration, the presence of diabetes mellitus, and past history of parathyroidectomy. For the risk factors of laboratory measures (serum corrected calcium, phosphate, calcium-phosphate product, eGFR, albumin, CRP, SBP, DBP, BMI, triglyceride, HDL-cholesterol, LDL-cholesterol, iPTH, HbA1c), the change in the values (2nd measured value – 1st measured value) were used. The other risk factors include current smoking status, statin use, and use of calcium inhibitors at baseline. In order to assess the non-linearity between the laboratory variables and the baseline Agatston score, non-linear restricted-cubic-spline was conducted in the regression model.

To consider whether the effects of these risk factors on 2nd Agatston score were modified by the baseline Agatston score (interaction), we performed similar multivariable linear regression analysis described above with a cross-product term between each risk factor and the baseline Agatston score. Adjustment for the baseline characteristics and non-linearity of the laboratory variables and baseline Agatston score were considered similarly to the linear regression analysis described above.

In these analyses, missing data were imputed with the multiple imputation method using “areg.impute” function in the “rms” package of R software (<https://cran.r-project.org/web/packages/rms/rms.pdf>).

All statistical inferences were made with 2-sided significance level of 5% and conducted with R software version 3.3.1 (<https://www.r-project.org/foundation/>) using the “rms” package.

Results

Baseline Characteristics of the Study Subjects (Table 1)

Table 1 presents the clinical and radiological characteristics of the 98 KTRs at baseline. The median age was 49 years, and 62.2% of the participants were men. The median dialysis duration, time from transplantation and estimated glomerular filtration rate (eGFR) were 3.4, 5.8 years and 48.5 mL/min/1.73 m², respectively. Diabetes mellitus, hypertension and dyslipidemia were found in 23.5, 88.8 and 33.7% of the participants, respectively. Of the participants, 6.2% had a smoking habit (current smoking). Of the 98 patients, AoC with an Agatston score >0 was detected in 77 (78.6%), and the remaining 21 (21.4%) exhibited no AoC (Agatston score = 0).

Change of the Agatston Scores (Fig. 1)

The medians of the baseline and 2nd Agatston scores were 713.9 (12.9–3,663.7), 999.8 (69.4–4,899.9), respectively. The 2nd Agatston scores were significantly greater than the baseline Agatston scores ($p < 0.001$). At the follow-up scan, which was performed after a median 3.2 (3.0–5.1) years, the prevalence of aortic calcification increased to 84.7% and individual Agatston scores progressed in 63.9% of patients. AoC increased by a median of 8.2 (4.0–20.8) % per year in KTRs with baseline Agatston score >30. The 2nd Agatston score was also 0 in 15 (71.4%) of the 21 KTRs with a baseline Agatston score of 0.

Fig. 1. Changes in the aortic calcification Agatston scores from the baseline CT to the 2nd CT scan.

Risk Factor Affecting the 2nd Agatston Scores (Table 2, Fig. 2)

After adjustment for confounders, the change in corrected serum calcium exhibited a significant non-linear correlation with the 2nd Agatston scores ($p = 0.022$ for non-linearity / $p = 0.031$ for effect of corrected serum calcium) (Table 2, Fig. 2). There were no significant correlations between the other clinical parameters and the 2nd Agatston scores (Table 2).

Interaction between the Change of Calcium and the Baseline Agatston Scores (Fig. 3)

The effect of corrected serum calcium on the 2nd Agatston scores was modified by the baseline Agatston score ($p = 0.049$), and the effect of hypercalcemia was greater in patients with higher baseline Agatston scores (Fig. 3).

Fig. 2. The effect of the change of calcium on the 2nd Agatston score.

Discussion

In the present study, we evaluated infrarenal abdominal AoC and examined the factors associated with its advancement in KTRs. As a result, AoC was prevalent in 78.6% of KTRs and the individual Agatston scores progressed in 63.9% of KTRs (Fig. 1). AoC increased by a median of 8.2% per year in KTRs with baseline Agatston scores >30. After adjustment for confounders, the change in corrected serum calcium exhibited a significant non-linear correlation with the AoC (Table 2 and Fig. 2). Moreover, an interaction was evident from the

Table 2. Risk factors affecting the 2nd Agatston scores

Variable (difference)	<i>p</i> value	<i>p</i> for non-linearity
Corrected calcium	0.022	0.031
Phosphate	0.975	0.975
Calcium × serum phosphorus product	0.929	0.961
i-PTH	0.991	0.897
Body mass index	0.12	0.084
eGFR	0.403	0.268
Calcineurin inhibitor	0.939	NA
Systolic blood pressure	0.583	0.759
Diastolic blood pressure	0.975	0.973
Total cholesterol	0.966	0.906
Triglycerides	0.275	0.138
High density lipoprotein cholesterol	0.512	0.315
Low density lipoprotein cholesterol	0.854	0.926
HbA1c	0.993	0.936
Serum albumin	0.815	0.652
C-reactive protein	0.759	0.716
Smoking habit	0.299	NA
Use of statin	0.671	NA

i-PTH, intact parathyroid hormone; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; NA, not available.

baseline AoC in the effect of corrected serum calcium on the progression of AoC, and the effect of hypercalcemia was greater in patients with more severe VC (Fig. 3). To our knowledge, this is the first report, in which infrarenal abdominal AoC was assessed quantitatively using the Agatston score and the risk factors for the progression of AoC were investigated using non-linear regression analysis in KTRs.

Most reported studies on VC in KTRs have investigated CAC using the Agatston score [11, 13–23], as described above, and reports on the AoC have been limited [12, 14, 16, 17]. Moreover, with respect to the evaluation of the AoC, the aortic segment measured has varied between studies and relative comparisons have been difficult. Four studies of KTRs using the Agatston score have been reported, but 2 and 1 of these examined thoracic AoC [14, 17] and descending AoC [16], respectively, and the remaining report examined total AoC [12]. Thus, our study initially evaluated infrarenal abdominal AoC. Studies that have evaluated aortic calcification using a semi-quantitative measurement method, such as the Aortic Calcification Index (ACI), have also occasionally been reported.

Many studies have reported the prevalence of CAC in KTRs to be 30–80%; although it has varied largely between the studies [11, 13, 14, 17, 19–22]. With respect to the prevalence of AoC in studies using the Agatston score, Nguyen et al. [17] and DeLoach et al. [12] reported prevalences of 85 and 33.9%, respectively; however, they evaluated thoracic AoC. In our study, the prevalence of AoC was 78.6%, which was close to the value reported by Nguyen et al. [17]. The frequency of AoC was lower in the study performed by DeLoach et al. [12], but the measurements were performed within 6 months after transplantation in all cases, which may explain the difference between their results and the findings of our study and those reported by Nguyen et al. [17] in which the subjects were examined years after transplantation.

Most studies on VC progression in KTRs have investigated CAC, and have concluded that progression of CAC slows down but does not halt after kidney transplant compared with dialysis patients [11, 13–15, 18–23, 28]. Moreover, with the increasing length of time after transplantation, worsening of VC was also observed [11, 13–15, 18–23, 28]. With respect to

Fig. 3. Interaction analysis suggested that the effect of exposure variable (the change volume of calcium) on the 2nd Agatston score were modified by the values of baseline Agatston scores. For example, we present the effect of corrected serum calcium at 3 pattern of baseline Agatston scores (10: 25th percentile, 800: 50th percentile, and 3,600: 75th percentile).

CAC, in studies that defined progression based on the SRC method [25], which was also used by ourselves, progression was observed in 23.2–34.5% of cases [11, 13, 20–22]. However, this should be interpreted carefully because the conditions, such as the observation period and timing of the study after transplantation, varied between the studies. To our knowledge, progression of AoC was only investigated using the Agatston score in 2 previous studies (Moe

et al. [16] and Maréchal et al. [14]). Moe et al. [16] showed no significant progression of AoC, which was likely a consequence of their small cohort ($n = 23$), the short follow-up period (15 to 20 months) and the timing of the investigation, which was immediately after transplantation (baseline CT scan was performed at the time of kidney transplantation). The study by Maréchal et al. [14] was the first to assess progression of AoC in a large population of KTRs ($n = 197$) with a relatively long follow-up (4.40 ± 0.28 years) and time following transplantation (8.3 ± 6.9 years). They observed that AoC increased by a median of 4% per year. In our study ($n = 98$, follow-up period; $3.2 [3.0–5.1]$ years, time from transplantation; $5.8 [2.8–10.0]$ years), AoC progression was observed in 63.9% of the patients when the SRC method was used, and the annualized rate percentage was 8.2% per year, suggesting that AoC also progresses substantially in KTRs. Progression was also observed following the evaluation of AoC using ACI [29, 30].

The annualized rate percentage of progression of infrarenal abdominal AoC in our study was about 2 times faster than that in the study on thoracic AoC reported by Maréchal et al. [14]. Maréchal et al. [14] simultaneously measured CAC and observed an annualized rate percentage of progression of 11% per year, suggesting that the calcification speed differs among regions, even in the same aorta.

Very few studies have reported risk factors of AoC progression in KTRs, and the study by Maréchal et al. [14] described above was the only one that used the Agatston score. Maréchal et al. [14] reported that baseline AoC, higher pulse pressure, statin use, older age, serum phosphate level, use of aspirin, and male sex were risk factors for AoC progression on multivariable analysis. However, since they used stepwise linear regression analysis for the multivariable analysis, it is possible that confounding factors were not adjusted for. In contrast, in our analysis, the baseline AoC, age, and sex were incorporated into the adjustment as unmodifiable factors, to adjust for confounders, and the amounts of changes in the modifiable factors were investigated as exploratory factors. In addition, non-linearity was taken into consideration in the analysis. As a result, a significant non-linear correlation with AoC progression was noted only in corrected serum calcium (Table 2 and Fig. 2). Although the study by Meneghini et al. [30] reported the evaluation of abdominal AoC using ACI, they showed that a high serum calcium level was a risk factor of AoC progression, which is consistent with our findings. While Maréchal et al. [14] extracted the serum phosphate level, higher pulse pressure, and statin therapy in their analysis, no significant differences were noted for any of these measures in our study. The reasons for the difference from our findings may be due to the location of the AoC. Reduction of the elastin/collagen ratio and elastin and collagen disarray are likely to occur in the abdominal aorta compared with those in the thoracic aorta [31, 32], and this degraded elastic fiber may cause calcium deposition [33]. Another possibility is that the difference may have been due to differences in the analytical method; thus, it is not possible to draw any definitive conclusions.

Moreover, serum calcium was not found to be a risk factor of CAC progression in any of the reports on risk factors of CAC [11, 13–15, 18–23, 28]. Although a combination of intimal and medial calcification may occur in patients with CKD, CAC is considered to be mainly due to intimal calcification, which is typically associated with atherosclerotic disease, and AoC to be due to medial calcification, which is mainly linked to mineral and bone disorders [34–36]. The influence of serum calcium on VC progression may readily appear in AoC due to region-associated difference in the mechanism of VC.

Many researchers have reported the presence of baseline VC as a risk of VC progression in KTRs [11, 13, 14, 21, 22]. On the other hand, no development of VC is observed, even at completion of follow-up, in most cases without VC at the baseline [11, 13, 14, 21, 22]. In our patients, the 2nd Agatston score was also 0 in 71.4% of patients with a baseline Agatston score of 0. Moreover, the effect of hypercalcemia on the progression of AoC was greater in

patients with more severe VC (Fig. 3). These findings suggest that management of hypercalcemia is particularly important for the prevention of calcification in CKD-MBD management of KTRs with severe VC.

Egbuna et al. [37] reported that KTRs with corrected calcium 10.5 mg/dL at one year after transplantation had a significantly higher risk of graft loss compared to those without. Thus, leaving hypercalcemia untreated can be harmful and hypercalcemia has previously been used as an index for CKD-MBD management after kidney transplantation, including management of tertiary hyperparathyroidism and criteria of therapeutic intervention [38]. As shown by our findings, these may be particularly appropriate from the perspective of VC.

The strengths of our study are as follows: (1) The prevalence and progression of infrarenal abdominal AoC, risk factors, and interactions were initially investigated using the Agatston score, (2) data were collected not only at the time of inclusion but also at the 2nd CT scan to investigate risk factors and the relationship between the value of change and progression based on the Agatston score, and (3) non-linear regression analysis, in which a non-linear association was considered, was used in the analysis of the Agatston scores, thus, showing greatly distorted data distribution.

The present study also has some limitations. Firstly, not all background factors could be adjusted for, because the sample size was small, which may have reduced the statistical power. A study with a larger sample size may be necessary in the future. In addition, with respect to the duration of follow-up, investigation for a longer follow-up period may be necessary. Secondly, only KTRs were investigated with no control group. A comparison of the frequency of AoC and risk factors between healthy subjects and CKD patients will be necessary. Thirdly, it is desirable to follow all patients from the initiation of kidney transplantation, because the risks of VC and arteriosclerosis vary depending on the time after kidney transplantation (e.g. due to the amount of immunosuppressive agents). Fourthly, we only investigated the infrarenal abdominal AoC. However, the relationships with CAC and AoC in other regions and differences in risk factors should be investigated.

In conclusion, the present study revealed that hypercalcemia was a risk factor for the development of infrarenal abdominal AoC in kidney transplantation recipients. Furthermore, the effect of hypercalcemia was greater in patients with more severe VC. Further studies are needed to explore whether infrarenal abdominal AoC predicts cardiovascular events and all-cause mortality in KTRs.

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Statement of Ethics

This study protocol was conducted in accordance with the Principles of the Declaration of Helsinki and the Declaration of Istanbul, and was approved by the ethics committee of the Osaka City University Graduate School of Medicine (No. 1415).

Disclosure Statement

The authors declare that they have no competing interests.

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Author Contributions

T.N. (corresponding author) contributed to the concept, design, data acquisition, interpretation, writing. D.K. and A.S. contributed to the data analysis and interpretation. Y.T., U.J. and T.N. reviewed and revised the manuscript. All authors read and approved the final manuscript.

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