HIGHLIGHTS

Validation of operational definitions of mortality in a nationwide hemodialysis population using the Health Insurance Review and Assessment Service databases of Korea

Glomerulonephritis following COVID-19 infection or vaccination: a multicenter study in South Korea

Role of APE1/Ref-1 in hydrogen peroxide-induced apoptosis in human renal HK-2 cells

Risk of mortality and cause of death according to kidney function parameters: a nationwide observational study in Korea

Prediction of diabetes mellitus after kidney transplantation using patient-specific induced pluripotent stem cells
Aims and Scope

*Kidney Research and Clinical Practice* (KRCP; formerly The Korean Journal of Nephrology; ISSN 1975-9460, launched in 1982), the official journal of the Korean Society of Nephrology, is an international, peer-reviewed journal published in English. Its ISO abbreviation is *Kidney Res Clin Pract*.

The journal considers articles on all aspects of nephrology and hypertension as well as molecular genetics, anatomy, pathology, physiology, pharmacology, and immunology related to kidney disease. In particular, the journal focuses on translational renal research that helps bridging laboratory discovery with the diagnosis and treatment of human kidney disease. The journal publishes the topics covered basic science with possible clinical applicability and the papers on the pathophysiological basis of the kidney disease. Original studies from areas of diagnostic and interventional nephrology or dialysis access are also welcomed. Major article types considered for publication include original research and reviews on current topics of interest.

To provide an efficient venue for dissemination of knowledge and discussion of topics related to basic research, translational study and clinical practice in nephrology, the journal offers online only open access, in which all published articles are free for everyone to read and download.

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Table of Contents

Editorial

131 On operational definitions of mortality
Hakmook Kang

Review Articles

133 Which blood pressure metrics should be used in patients on dialysis?
Ji Yong Jung

143 Pharmacologic therapeutics in sarcopenia with chronic kidney disease
Ran-hui Cha

Original Articles

156 Validation of operational definitions of mortality in a nationwide hemodialysis population using the Health Insurance Review and Assessment Service databases of Korea
Dong Hee Lee, Ye-Jee Kim, Hyangkyoung Kim, Hyung Seok Lee

165 Glomerulonephritis following COVID–19 infection or vaccination: a multicenter study in South Korea
Hyung Woo Kim, Eun Hwa Kim, Yun Ho Roh, Young Su Joo, Minseob Eom, Han Seong Kim, Mi Seon Kang, Hoeln Jeong, Beom Jin Lim, Seung Hyeok Han, Minsun Jung; Renal Pathology Study Group of Korean Society of Pathologists

177 Vitamin D and narrowband ultraviolet B phototherapy for chronic kidney disease–associated pruritus
Youn Kyung Kee, Hee Jung Jeon, Jieun Oh, Dong Ha Shin

186 Role of APE1/Ref–1 in hydrogen peroxide–induced apoptosis in human renal HK–2 cells
Ha Yeon Kim, Jung Sun Park, Byeong Hwa Jeon, Hong Sang Choi, Chang Seong Kim, Seong Kwon Ma, Soo Wan Kim, Eun Hui Bae

202 Risk of mortality and cause of death according to kidney function parameters: a nationwide observational study in Korea
Sehyun Jung, Soojin Lee, Yaerim Kim, Semin Cho, Hyuk Huh, Yong Chul Kim, Seung Seok Han, Hajeong Lee, Jung Pyo Lee, Kwon Wook Joo, Chun Soo Lim, Yon Su Kim, Dong Ki Kim, Kyungdo Han, Sehoon Park

216 A collaborative model between dialysis clinics and a hospital center improves the quality of vascular access care and intervention for hemodialysis patients
Chung-Kuan Wu, Yu-Wei Fang, Chia-Hsun Lin

226 Circulatory endostatin level and risk of cardiovascular events in patients with end–stage renal disease on hemodialysis
Jin Sug Kim, Miji Kim, Kyung Hwan Jeong, Ju-Young Moon, Sang Ho Lee, Gang Jee Ko, Dong-Young Lee, So Young Lee, Yang Gyun Kim, Hyeon Seok Hwang
Prediction of diabetes mellitus after kidney transplantation using patient-specific induced pluripotent stem cells

Sun Woo Lim, Yoo Jin Shin, Sheng Cui, Eun Jeong Ko, Byung Ha Chung, Chul Woo Yang

Images in Practice

Renal infarction caused by spontaneous renal artery dissection after playing golf

Seung Hee Jeong, Dong Min Kang, Ju Hwan Oh, A Young Cho, In O Sun, Kwang Young Lee, Haeun Lee

Correspondence

Rapidly progressive glomerulonephritis in the elderly: a case of cryoglobulinemic glomerulopathy not to be overlooked

Hyeran Park, Seyoung Ryou, Seung Yun Chae, Eun Ah Kim, Jong-Mi Lee, Yaeni Kim, Yeong-Jin Choi, Cheol Whee Park
Mortality or mortality rate has been collected since 1750's and are utilized across various research fields such as epidemiology, biostatistics, and biomedical and biopharmaceutical research. Excess mortality, denoting mortality above the normal rate, typically demands deeper investigation as it can signal fatal diseases or infections. In biomedical research, unraveling the causes of excess mortality often sparks new lines of inquiry, such as research into developing vaccines for coronavirus disease 2019. Consequently, instances of increased mortality or excess mortality consistently attract significant attention in the biomedical research realm.

In the era of big data, encompassing electronic health records (EHRs), multi-modal magnetic resonance imaging data, and multiomics data, comprehending data structures and integrating diverse information sources to address critical scientific questions is paramount. In large-scale datasets, encountering missing data is inevitable due to various factors, such as randomness or systematic issues. Particularly, EHR-type data tend to exhibit missing observations.

One strategy to tackle the missing data challenge involves imputing missing values using non-missing information within the dataset, provided the non-missing information predicts the missing values. For example, missing body weight values could be imputed using height, sex, and age data via a linear regression model. Herein, understanding variable associations within a dataset is pivotal for successful imputation.

Nevertheless, when missing observations arise from systematic missingness, such as the absence of death information in the Health Insurance Review and Assessment Service database, imputation becomes more limited and complex. Integrating a dataset containing systematic missingness with another containing the missing information can help obtain the necessary data without constructing an imputation model. In cases where obtaining another dataset with the missing information isn’t feasible, imputation methods such as single or multiple imputation may need to be considered.

Mortality stands as a cornerstone parameter in biomedical research. Sometimes, addressing critical scientific inquiries becomes infeasible without knowledge of mortality rates. In such instances, operational definitions of mortality, as outlined in, can be established. A recent article by Lee et al. titled “Validation of operational definitions of mortality in a nationwide hemodialysis population using the Health Insurance Review and Assessment Service databases of Korea,” delves into the validation of several operational definitions of mortality. These defi-
nitions encompass intervals of 30, 60, 90, 120, 150, and 180 days of no health insurance claims. The study reveals that an operational definition requiring 150 days free from health insurance claims yielded the most accurate results.

From a statistical standpoint, the uniformity of a single definition across all age and sex groups is intriguing. Nonetheless, leaning towards more conservative or lenient mortality estimates based on a single operational definition within a particular age group could introduce bias to study outcomes. Employing statistical models to account for potential confounding factors like age or sex might be essential. If one definition consistently overestimates mortality within a specific age and sex combination, proposing an alternative definition for that combination could prove beneficial. Similarly, suggesting multiple operational definitions for various strata, such as specific age and sex combinations, could help mitigate bias. However, the trade-off between bias and variance needs careful consideration, as too many definitions may lead to overfitting and reduced generalizability.

The assessment of estimation can be approached through external and internal validations. While external validation using external datasets is the gold standard, internal validation can be achieved through k-fold cross-validation combined with bootstrapping when external data are unavailable.

In this context, exploring the distribution of deviations from true values for each age stratum via bootstrapping could be illuminating. Repeatedly resampling data within each age group and calculating deviations from true values generates a bootstrap distribution of deviations, essentially reflecting the variation of mortality within each age group. By proposing multiple operational definitions and generating corresponding bootstrap distributions of deviations, it becomes possible to identify approaches that exhibit the smallest variance, thus being more generalizable to similar datasets. Beyond proposing definitions, assessing the variance associated with each definition contributes to finding an optimal balance between bias (measured by mean deviation) and variance (bootstrap variance).

Conflicts of interest

The author has no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

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References

Which blood pressure metrics should be used in patients on dialysis?

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Remarkable progress has recently been achieved in blood pressure (BP) control based on key research findings in the general population. It has been observed that maintaining BP slightly lower than previously recommended goals leads to better clinical outcomes, provided that patients can tolerate it. Previously, BP control targets for dialysis patients were extrapolated from studies conducted on the general population. However, dialysis patients are considered a distinct group with unique characteristics, which makes defining appropriate BP targets a matter of debate. Several observational studies measuring BP in hemodialysis (HD) patients within dialysis units have shown that lower peridialysis BP (pre-, post-, and interdialytic BP) is associated with worse clinical outcomes. However, this association is likely confounded by factors specific to dialysis patients. The relationship between BP and mortality appears to be more linear in patients with fewer underlying cardiovascular diseases and longer survival. Recent studies have indicated that BP measurements taken outside of dialysis sessions, such as standardized BP on nondialysis days, home BP, and ambulatory BP monitoring between HD sessions, are more predictive of clinical outcomes. Due to the varied effects of dialysis-related treatment practices on BP, there is a lack of data from large-scale clinical trials. As a result, it is challenging to provide strong recommendations for BP targets directly applicable to dialysis patients. This review addresses various factors influencing BP in dialysis patients, including the establishment of individualized target BP levels and discussions on maintenance strategies, while incorporating a recent literature review.

Keywords: Blood pressure determination, Dialysis, Risk assessment

Introduction

Hypertension is a very common clinical condition among chronically ill patients and is one of the important factors in end-stage kidney disease (ESKD) [1-4]. In addition, blood pressure (BP) status long-term hemodialysis (HD) is often not properly diagnosed and is poorly controlled [5-7]. Moreover, the recent coronavirus disease 2019 pandemic has served as an opportunity to confirm once again how difficult it is to maintain public health while consuming many social resources [8-10].

Unlike the linear relationship between BP and mortality in the general population [11,12], the inverse or U-shaped association between pre- and postdialysis BP and mortality in HD patients is important for controlling hypertension and setting target BP in HD patients [13,14]. In contrast, there are consistent reports that elevated BP, as assessed by home BP recording or ambulatory BP (ABP) monitoring...
between dialysis sessions, provides direct and unequivocal signs of death [15,16]. These results suggest that the patient’s underlying disease and comorbidities affect dialysis-related BP fluctuations and consequently affect clinical outcomes. As in the general population, further research is needed on whether maintaining tolerably low BP in dialysis patients is associated with positive clinical outcomes.

Although it is difficult to determine the exact level of BP to target in HD patients, a meta-analysis of randomized trials reports that lowering BP using antihypertensive therapy improves clinical outcomes, particularly in hypertensive patients [17]. In addition, in the case of HD patients, it is possible to administer antihypertensive drugs together with nonpharmacological strategies such as sodium and body fluid control in order to stably maintain HD [18–22]. Through this strategic therapeutic combination, an appropriate BP for each individual patient should be targeted and maintained.

In this review, the author has considered multiple factors affecting BP in HD patients, establishing individualized target BP levels and discussing strategies for maintenance, accompanied by a recent literature review.

Blood pressure targets in the general population and chronic kidney disease

The SPRINT (Systolic Blood Pressure Intervention Trial) is considered to be one of the studies that have the greatest impact on target BP change in the recent clinical practice guidelines for BP control. A total of 9,631 nondiabetic patients at relatively high risk of cardiovascular (CV) event were enrolled, and their effects on CV, renal, and mortality outcomes were investigated in a control group with a target systolic BP (SBP) of <140 mmHg and an intensive group with a target SBP of <120 mmHg [23]. They reported a 25% reduction in the primary composite outcome of CV mortality and morbidity in those randomized to the intensive group (hazard ratio [HR], 0.75; 95% CI, 0.53–0.99) [24]. In addition, the result of subgroup analysis targeting only chronic kidney disease (CKD) patients showed a similar all-cause mortality reduction effect (HR, 0.72; 95% CI, 0.53–0.99) [24].

In contrast, in the ACCORD-BP (Action to Control Cardiovascular Risk in Diabetes-BP) trial, the target SBP of <120 mmHg group was compared with the target SBP of <140 mmHg in 4,733 diabetic patients with serum creatinine concentration of ≤1.5 mg/dL, but there was no reduction effect in the primary CKD composite outcome (HR, 0.88; 95% CI, 0.73–1.06) [25]. However, among the prespecified secondary outcomes, a reduction in stroke incidence was reported (HR, 0.59; 95% CI, 0.39–0.89) [25].

As a result of the AASK (African American Study of Kidney Disease and Hypertension) trial in 1,094 nondiabetic hypertensive African Americans, among patients with a protein-to-creatinine ratio of 0.22 g/g or higher, patients in the intensive group (target mean BP, 92 mmHg), compared to the control group (target mean BP, 102–107 mmHg), showed a reduction in the incidence of ESKD or death (HR, 0.67; 95% CI, 0.52–0.87) [26].

In the STEP (Strategy of BP Intervention in the Elderly Hypertensive Patients) trial with 8,511 relatively elderly (60 to 80 years of age) Chinese patients, the intensive treatment group (target SBP, 110–130 mmHg), compared with the control group (target SBP, 130–150 mmHg), showed a reduction effect on primary CV (HR, 0.74; 95% CI, 0.60–0.92), but the renal outcome as a secondary outcome (>50% reduction of estimated glomerular filtration rate in CKD patients at baseline) showed no reduction effect (HR, 1.01; 95% CI, 0.66–1.60). In addition, the risk of developing hypertension according to the BP-lowering effect of the intensive treatment group was also reported to be high (HR, 1.31; 95% CI, 1.02–1.68) [27].

Recently, different organization guidelines [28–30] have set the target BP somewhat lower. However, since this target BP is a value measured by standardized BP equipment, it tends to be difficult to apply in the outpatient clinic environment in Korea. Therefore, the Korean Society of Hypertension sets the target value somewhat higher in consideration of conventional BP measurement variations [31,32].

Blood pressure and outcomes in dialysis patients

The 2005 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines recommend pre- and postdialysis BP goals of 140/90 and 130/80 mmHg, based in part on data in the nondialysis population [33]. However, most previous studies have shown a J- or U-shaped association, which appears to increase mortality when SBP is low [13,14].

In the DOPPS (Dialysis Outcomes and Practice Patterns in
Study), which enrolled 25,907 HD patients in 922 facilities, predialysis SBP and diastolic BP (DBP) were set and analyzed with 130 to 139 mmHg and 80 to 89 mmHg, respectively, as reference groups, and it was found that HR increased in the BP reduction group [13].

In a study that analyzed all-cause mortality over 548 days in a prospective study of 9,333 HD patients in France, the association between predialysis SBP and DBP and all-cause mortality showed also a U-shaped association that the lowest risk in that study was observed at around 165 mmHg [14].

As a result of a domestic study that analyzed 2,299 patients receiving dialysis (both HD and peritoneal dialysis [PD]) from the CRC-ESRD (Clinical Research Center for End-Stage Renal Disease) data, a U-shaped relationship was reported between SBP and mortality during the median follow-up period of 4.5 years [34]. The lowest risk was shown in the groups with 130 to 150 mmHg of SBP. When the continuous BP was categorized, the group with SBP under 110 mmHg and the group with SBP higher than 170 mmHg showed an increased HR for mortality during follow-up [34]. The lowest SBP was the only risk factor for death in the elderly and those with diabetes or coronary artery disease, whereas the highest SBP was the only risk factor in younger people [34]. A U-shaped association was seen in patients undergoing HD (this association was not seen in PD patients), and among HD patients, a U-shaped association was more pronounced when the dialysis period was short and the weight gain during dialysis was small [34].

As for the reasons why the relationship between BP and mortality was different in the dialysis group compared to nondialysis patients, serious coexisting conditions in HD patients and confounding due to poor health status were suggested [13,18–21,35,36].

The relationship between blood pressure and mortality in hemodialysis patients varies depending on the timing of observation or the presence of comorbidity

A study of a cohort of HD patients (n = 16,959) in the United States between January 1, 1993, and December 31, 2003, showed that low SBP of <120 mmHg during the first 2 years of follow-up was associated with an increased mortality rate, and only patients who survived more than 3 years were analyzed, and a SBP of ≥150 mmHg was associated with increased mortality [37]. These data suggest the possibility that high mortality in the low BP group may be confounding due to underlying disease and not a causal risk factor for adverse outcomes.

As a result of analyzing 6,585 prevalent HD patients randomly selected from the United States Renal Data System data, the low BP group showed a higher mortality rate only in the comorbidity group such as congestive heart failure or coronary artery disease [38]. However, the increase in mortality in the hypotension group was not significant in the group without comorbidities [38].

In a study in which 344 HD patients (105 with atrial fibrillation, heart failure, or both) were analyzed by measuring 24-hour ABP starting before the dialysis session [39]. In a linear subgroup analysis, SBP and pulse pressure were independent predictors of risk and showed a significant inverse association with all-cause and CV death in patients with atrial fibrillation or heart failure [39]. However, in patients without these comorbidities, these associations were in the opposite direction [39]. Furthermore, it suggests that the associations can be explained by underlying cardiac disease. These findings support the importance of considering the comorbidity of cardiac disease when treating hypertension in HD patients. The authors claim that this opposite linear association can be explained by the presence or absence of underlying heart disease, further supporting the importance of considering the comorbidity of heart disease when treating hypertension in HD patients [39].

Our society is rapidly transitioning from an “aged society” to a “super-aged society,” highlighting the significance of maintaining CV health as part of our efforts to safeguard the well-being of the growing elderly population [40]. This issue is of great importance not only for dialysis patients but also for the general elderly population. For frail individuals, including those with severe comorbidities or advanced age, providing immediate and intensive medical support, such as determining the optimal timing to initiate dialysis and target BP, can significantly reduce premature mortality [41]. Therefore, it is crucial to consider the prudent utilization of our societal resources in addressing this matter collectively.
**Time points of blood pressure measurement on the outcomes in hemodialysis patients**

**Dialysis-unit blood pressure measurement**

The BP behaviors of HD patients presented a unique pattern much different from those of the general population. This pattern is composed of chronic BP burden over interdialytic period and acute BP fluctuation during HD sessions. Peri-, inter-, and intradialysis are three routinely used time points to capture this complex BP behavior [42]. However, BP at each time point was measured in various forms and conveyed different prognostic information [42]. There has been debate as to which BP metrics should be used, both in studies and in clinical practice (Fig. 1).

SBP decreased by an average of 8 to 10 mmHg after HD, showing great differences among patients and individuals [42]. As a result of a meta-analysis comparing ABP and predialysis BP in patients who performed HD three times a week, predialysis BP tended to be higher than ABP, while postdialysis BP tended to be low [43].

Single-point measurement of BP is insufficient to evaluate prognosis in HD patients. As a result of 35 months of follow-up by measuring average weekly BP and pulse pressure, it has been reported that mean BP can be a better prognostic indicator than single-point BP measurement for the incidence of CV events and all-cause mortality in HD patients [44].

Some researchers suggest that the ultimate BP target for all HD patients should include targets for peridialytic, interdialytic, and intradialytic BP, not a single target [42].

**Out-of-dialysis blood pressure measurement**

Home blood pressure (HBP) monitoring is widely applied and strongly recommended for the diagnosis and management of hypertension in the general population in current international and domestic clinical practice guidelines [28,29,32]. These HBP monitoring may be particularly useful in HD patients than in any other patient population. While the agreement between peridialytic BP and interdialytic ABP was poor, average self-measured systolic HBP was useful for detecting hypertension between dialysis using 44-hour ABP monitoring as a reference [45]. While short-term variability in BP records before and after HD is high [46], HBP is highly reproducible as a result of weekly measurement [47]. HBP is also superior to dialysis-unit-BP in predicting future risk of CV and mortality [16,48].

To date, interdialytic ABP monitoring is considered as the gold standard for BP measurement among HD patients [48,49]. ABP levels between dialysis are highly reproducible [33], correlate with left ventricular hypertrophy [50], and show a direct correlation with mortality [16,48]. The advantage of this method is not only that the number of ABP measurements is performed more frequently but also that it can reflect a wider range of situations and activities in

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**Figure 1. Time point of BP measurement in HD patients.**

ABP, ambulatory BP; BP, blood pressure, HD, hemodialysis.
the patient’s living environment related to dialysis so that the patient’s general BP burden can be more accurately reflected [16,18,20,22,48]. In addition, while dialysis-unit BP measurement cannot avoid the white coat effect, in which BP is high only on dialysis and normal BP outside of HD, this effect can be easily confirmed by self-measured HBP or ABP measurement [16,48,49].

ABP monitoring between dialysis provides very powerful prognostic information, but it is impractical to continuously use this measurement method for hypertension management in HD patients. This has been a practical reason for a long time in the past that BP goal setting and overall management of hypertension were based on relatively easily available pre- and postdialysis BP records. Therefore, as a result of a recent study, it is considered realistic to use dialysis-unit BP as an auxiliary measure for the maintenance of dialysis and to use HBP monitoring as a main tool for managing hypertension in the long term. In some cases, physicians may consider using ABP monitoring individually for a specific purpose on a patient (Fig. 1).

Although it is a reality that research results for presenting target BP in HD patients are lacking, lowering interdialytic BP could be clinically beneficial, and for this purpose, proposed BP target in the study by Alborzi et al. [16] might be referred to as a target (ABP monitoring, 115–125 mmHg; HBP monitoring, 125–145 mmHg) for HD patients in clinical practice.

Pertinent information from the analysis of systematic review and meta-analysis data

There have been clinical trials on the usefulness of various antihypertensive agents in HD patients. As a result of a meta-analysis of eight studies [17], it was reported that the treatment group showed an effective BP-lowering effect compared to the control group, and showed an effect of reducing CV (HR, 0.71; 95% CI, 0.50–0.99) and all-cause (HR, 0.80; 95% CI, 0.66–0.96) mortality. In another meta-analysis of 1,202 patients from five studies [51], compared with control or placebo groups, the overall benefit of antihypertensive therapy showed the effect of reducing CV events in a fixed-effects model (HR, 0.69; 95% CI, 0.56–0.84) and random-effects models (HR, 0.62; 95% CI, 0.45–0.86). In particular, in their sensitivity analysis, the CV protective effect shown in the result of analyzing only the hypertensive group (HR, 0.49; 95% CI, 0.35–0.67) was not seen as that of the normal BP group (HR, 0.86; 95% CI, 0.67–1.12).

Some traits necessitate a careful interpretation of the study results for lowering BP in HD patients because the BP reduction achieved by patients varied widely among the trials, and the baseline BP level was heterogeneous in each study. Moreover, most randomized clinical trials were based on a specific drug, not a target BP. This made it difficult to pool BP targets when the Korean Society of Nephrology (KSN) Clinical Practice Guideline Work Group systemically reviewed the literature on BP targets of HD patients for guideline development [52,53]. Therefore, the evidence is insufficient to determine whether the effect of antihypertensive medication is a drug-specific effect or a result of reducing the BP below a certain threshold.

Noteworthy insights from the clinical trial data

A study that randomly assigns HD patients to different BP targets has not yet been conducted, and BID (Blood Pressure in Dialysis) trial is the only recent pilot study that has evaluated the possibility of a full-scale study [54]. A total of 126 patients who had been on HD for more than 3 months and had a 2-week average predialysis SBP higher than 155 mmHg were evaluated for intensive arm (110–140 mmHg, n = 62) or standard arm (155–165 mmHg, n = 64). They were randomly assigned to the predialysis SBP targets and observed for 1 year. The primary outcomes of the study were to assess the feasibility and safety of treating hypertensive patients receiving HD and to inform the design of a full-scale study and assessing changes in left ventricular mass was a secondary outcome. During months 4 to 12 the average difference in SBP across arms was 12.9 mmHg. There was no significant difference number of follow-up loss between the two groups during observation, ultimately 51 patients in the intensive group and 50 patients in the standard group were followed up. There was an increase in hospitalizations and vascular access thromboses in the intensive arm, but given the small size and relatively short follow-up time, these were not statistically significant. The incidence rate ratios for the intensive compared with the standard arm (95% CI) were 1.18 (0.40–3.33), 1.61 (0.87–2.97), and 3.09 (0.96–8.78) for major adverse CV events, hospitalizations, and vascular access thrombosis, respectively. The intensive and standard arms had similar
median changes (95% CI) in left ventricular mass of 20.84 g (217.1–10.0 g) and 1.4 g (211.6–10.4 g), respectively. The authors noted that, although it was not statistically significant, there was a tendency to decrease left ventricular mass [54]. Due to the small number of enrolled population and the possibility of being associated with a short observation period, they could not come to a conclusion whether the active control of predialysis BP had a long-term effect on left ventricular mass and ultimately led to an improvement in mortality [18,21,54]. Therefore, the authors suggested the need for a full-scale study to determine definitely the effects of intensive BP control on clinical outcomes in HD patients.

Potential harms of blood pressure reduction in hemodialysis patients

To examine the relationship between the achieving target BP (predialysis BP of 140/90 mmHg and postdialysis BP of 130/80 mmHg) recommended by the United Kingdom Renal Association and CV risk, 7,890 HD sessions for 1 week were analyzed in 2,630 patients [55]. As a result, the achieving rate of the target pre- and postdialysis BP were 36% and 42%, respectively, with large differences between centers. About 15% of patients showed symptomatic hypotension requiring fluid resuscitation, and it was reported that the higher achievement rate of the target postdialysis BP, the higher the incidence of hypotension during HD [55].

As a result of a Japanese study that analyzed risk factors for intradialytic hypotension in 111 HD patients, diabetes mellitus, excessive interdialytic weight gain, low ejection fraction, and low left ventricular volume were analyzed as independent risk factors for hypotension during HD [56]. However, no correlation was found between predialysis SBP values or the addition of antihypertensive medications and the incidence of intradialytic hypotension [56].

In clinical practice, there are HD patients who need to technically decide when or whether to continue taking or temporarily holding BP medications to maintain HD. There is a study that can serve as a reference for this regard (TAKE-HOLD trial). Compared to the holding group on the day of dialysis (HOLD), the group taking antihypertensive drugs daily (TAKE) did not show an inferior outcome in intradialytic hypotension and reduced the incidence of uncontrolled hypertension [57]. The authors concluded that the question remains as to whether the reduction in the incidence of intradialytic hypotension with the strategy of holding BP medication is an offset effect due to elevated predialysis BP [57]. The TAKE-HOLD strategies could be applied to patients with intradialytic hypotension. Therefore, if additional studies on those patients were conducted in the future, the effect of holding BP medication before dialysis would provide more useful information to clinicians who are struggling between maintaining stable HD and controlling interdialytic BP.

Generally, it is highly likely to cause intradialytic hypotension in frail patients, such as those with underlying comorbidities and/or accompanying sarcopenia [58]. In this case, the patient’s exercise power can help lower BP and improve quality of life [59].

The current guidelines regarding blood pressure measurement and targets in hemodialysis patients

Most of the recommendations regarding target BP for dialysis patients in the various guidelines are based on expert opinion, as existing observational and clinical studies are notably lacking. The 2005 KDOQI guidelines recommended predialysis BP targets of <140/90 mmHg and postdialysis BP of <130/80 mmHg (Table 1) [33]. However, these recommendations were given a “C” rating for their strength, as they were extracted from studies in the general population and there was no clinical trial-level evidence [33]. Additionally, the 2015 update of the guideline stated that there was insufficient evidence to support a specific BP target and did not suggest a target BP [60].

In the Canadian Society of Nephrology in 2006 [61] and Japan Society for Dialysis Therapy in 2012 [62], predialysis BP of 140/90 mmHg or higher was suggested as hypertension (Table 1).

In the relatively recent European Renal Association-European Dialysis and Transplant Association (ERA-EDTA)/European Society of Hypertension (ESH) guidelines [63], peridialysis BP measurement is not recommended, and the definition of hypertension was suggested that a case where the average value of home BP measured over 6 days on nondialysis days was ≥135/85 mmHg or the average value of ABP measured for 44 hours on a weekday was ≥130/80 mmHg. In addition, when neither HBP nor ABP monitor-
Table 1. Definition of hypertension in patients on dialysis according to clinical practice guidelines

<table>
<thead>
<tr>
<th>Guidelines</th>
<th>Year</th>
<th>Definition</th>
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<tr>
<td>KDOQI 2005</td>
<td>2005</td>
<td>• Predialysis BP of &gt;140/90 mmHg</td>
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<tr>
<td></td>
<td></td>
<td>• Postdialysis BP of &gt;130/80 mmHg</td>
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<tr>
<td>KDOQI 2015 update</td>
<td>2015</td>
<td>• No target defined, citing paucity of clinical trial data</td>
</tr>
<tr>
<td>CSN 2006</td>
<td>2006</td>
<td>• Predialysis BP of &gt;140/90 mmHg</td>
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<tr>
<td>JSDT 2012</td>
<td>2012</td>
<td>• Predialysis BP of &gt;140/90 mmHg at the beginning of the week (without cardiac dysfunction)</td>
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<tr>
<td>ERA-EDTA/ESH 2017</td>
<td>2017</td>
<td>• No recommendation can be made on the basis of peridialytic BP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Home BP: an average BP of ≥135/85 mmHg for measurements collected in the morning and in the evening over 6 nondialysis days (covering a period of 2 weeks)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ABP: an average BP of ≥130/80 mmHg over 24-hr monitoring during a mid-week day free of HD. Whenever feasible, ABP monitoring should be extended to 44 hours, that is, covering a whole mid-week dialysis interval</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Office BP of ≥140/90 mmHg taken in a mid-week day free of HD (when neither ABP nor home BP measurements are available)</td>
</tr>
<tr>
<td>KSN 2021</td>
<td>2021</td>
<td>• Inconclusive, insufficient evidence to assign optimal BP target for HD patients.</td>
</tr>
</tbody>
</table>

ABP, ambulatory blood pressure; BP, blood pressure; CSN, Canadian Society of Nephrology; ERA-EDTA, European Renal Association-European Dialysis and Transplantation Association; ESH, European Society of Hypertension; HD, hemodialysis; JSDT, Japan Society for Dialysis Therapy; KDOQI, Kidney Disease Outcomes Quality Initiative; KSN, Korean Society of Nephrology.

Table 2. Proposed approaches for BP management in HD patients

<table>
<thead>
<tr>
<th>Methods</th>
<th>Opinions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home blood pressure</td>
<td>• All patients</td>
</tr>
<tr>
<td></td>
<td>• BP measurement twice daily for 7 days</td>
</tr>
<tr>
<td></td>
<td>• Systolic BP, 120–135 mmHg</td>
</tr>
<tr>
<td></td>
<td>• Diastolic BP, 60–80 mmHg</td>
</tr>
<tr>
<td>Office BP on a nondialysis day</td>
<td>• Alternative (when HBP is not available)</td>
</tr>
<tr>
<td></td>
<td>• Systolic BP, 140 mmHg</td>
</tr>
<tr>
<td></td>
<td>• Diastolic BP, 60–80 mmHg</td>
</tr>
<tr>
<td>Dialysis-unit BP</td>
<td>• Predialysis systolic BP, 130–159 mmHg</td>
</tr>
<tr>
<td></td>
<td>• Predialysis diastolic BP, 60–99 mmHg</td>
</tr>
<tr>
<td></td>
<td>• Postdialysis systolic BP, 120–139 mmHg</td>
</tr>
<tr>
<td></td>
<td>• Postdialysis diastolic BP, 70–89 mmHg</td>
</tr>
<tr>
<td>44-hr Ambulatory BP</td>
<td>• BP is not at the target</td>
</tr>
<tr>
<td></td>
<td>• When the patients have intradialytic hypotension or hypertension</td>
</tr>
</tbody>
</table>

BP, ambulatory blood pressure; HD, hemodialysis.

In order to properly manage BP in HD patients, appropriate dialysis, volume management, medication, patient factor control, etc. should be considered as an integrated and strategic approach. According to experts’ opinions at the 2020 KDIGO (Kidney Disease: Improving Global Outcomes) controversies conference, BP control requires a multidisciplinary approach considering volume management, and numerous strategies and technologies to be considered in the design and implementation of future clinical trials in an area that has not yet been sufficiently studied have been suggested [64].

BP targets in HD patients are uncertain due to the lack of clinical trial data for guidance and uncertainties in observational data. Recognizing the increasing association of out-of-dialysis BP (HBP and ABP) with clinical outcomes in patients with ESKD, we also need to acknowledge the cumbersomeness and limitations of those measures. One
approach to integrating HBP into the management of hypertension in HD patients has the potential to improve BP control, involve patients in treatment planning, and better predict hypertension outcomes. Until clinical trials that present clear clinical evidence are provided in the future, it is thought that it is best to facilitate patient BP control using all available means such as HBP and ABP rather than using only BP at the HD clinic to manage hypertension. As one of these efforts, it is possible to check the BP measurement and target BP of HD patients suggested as the experts’ opinions and to use it as a reference during clinical treatment (Table 2) [20].

Opinions regarding blood pressure measurement and targets in peritoneal dialysis patients

Compared to HD patients, there are few clinical studies that show clear evidence worldwide, but since the dialysis-related BP fluctuation is small, it can be controlled with the same management strategies as nondialysis patients. As in the case of HD, there are no guidelines for target BP for PD patients. However, the joint working group ERA-EDTA and the ESH suggested the diagnosis of hypertension in PD patients was an average value of home BP for 7 days higher than 135/85 mmHg and a 24-hour average value of ABP monitoring higher than 130/80 mmHg [63].

Conclusions

In the case of HD patients, mortality due to CV complications is high, and BP control is important as one of the efforts to prevent it. Clinical guidelines for BP treatment for the general population are continuously evolving, but in the case of dialysis patients (both HD and PD), due to the absence of clinical trials, guidelines for dialysis patients are extrapolated and applied from research results and practice guidelines for the general population. Therefore, strong recommendations for BP targets for dialysis are difficult to apply directly to patients.

A low correlation between dialysis-unit BP measurement and clinical outcomes has been reported in the HD population, and the studies reporting the importance of HBP and ABP measurement are increasing. Therefore, from a practical point of view, BP control can be attempted using dialysis-unit BP and HBP until future evidence studies are conducted and practical information is provided. As a strategic approach to control, it is necessary to set an appropriate target BP for individual patients and establish a strategy to maintain it.

Conflicts of interest

The author has no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available on request from the corresponding author.

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References


Pharmacologic therapeutics in sarcopenia with chronic kidney disease

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Introduction

Sarcopenia is characterized by the loss of muscle mass and strength, decreased physical performance, decreased quality of life (QoL), morbidities, and immobility with age, and it is associated with protein-energy wasting in chronic kidney disease (CKD) and end-stage kidney disease (ESKD) patients [1]. There have been several recommendations for sarcopenia criteria in Europe and Asia (Table 1) [2,3]. These recommendations use muscle strength, muscle quantity or quality, and physical performance to diagnose and classify sarcopenia.

Sarcopenia is primarily the age-related progressive loss of muscle mass and strength (muscle atrophy). Uremic sarcopenia means a progressive decrease in muscle mass, strength, and function despite normal skeletal muscle

Inflammation, metabolic acidosis, renin-angiotensin system activation, insulin resistance, and impaired perfusion to skeletal muscles, among others, are possible causes of uremic sarcopenia. These conditions induce the activation of the nuclear factor-kappa B and mitogen-activated protein kinase pathways, adenosine triphosphate ubiquitin-proteasome system, and reactive oxygen species system, resulting in protein catabolism. Strategies for the prevention and treatment of sarcopenia in chronic kidney disease (CKD) are aerobic and resistance exercises along with nutritional interventions. Anabolic hormones have shown beneficial effects. Megestrol acetate increased weight, protein catabolic rate, and albumin concentration, and it increased intracellular water component and muscle mass. Vitamin D supplementation showed improvement in physical function, muscle strength, and muscle mass. Correction of metabolic acidosis showed an increase in protein intake, serum albumin levels, body weight, and mid-arm circumference. The kidney-gut-muscle axis indicates that dysbiosis and changes in gut-derived uremic toxins and short-chain fatty acids affect muscle mass, composition, strength, and functional capacity. Biotic supplements, AST-120 administration, hemodiafiltration, and preservation of residual renal function are alleged to reduce uremic toxins, including indoxyl sulfate (IS) and p-cresyl sulfate (PCS). Symbiotics reversed the microbiota change in CKD patients and decreased uremic toxins. AST-120 administration changed the overall gut microbiota composition in CKD. AST-120 prevented IS and PCS tissue accumulation, ameliorated muscle atrophy, improved exercise capacity and mitochondrial biogenesis, restored epithelial tight junction proteins, and reduced plasma endotoxin levels and markers of oxidative stress and inflammation. In a human study, the addition of AST-120 to standard treatment had modest beneficial effects on gait speed change and quality of life.

Keywords: Chronic kidney disease, Drug, Sarcopenia, Treatment
physiology in CKD patients. Uremic sarcopenia is not solely associated with the advanced age. It is mainly associated with the characteristics of uremia, such as metabolic acidosis, chronic inflammation, vitamin D deficiency, insulin resistance, hormonal changes, and gut dysbiosis, which contribute to the development of increased protein catabolism and reduced protein synthesis.

I will deal with the clinical significance, prevalence, risk factors, and strategies to prevent and treat sarcopenia with CKD in this review.

**Clinical significance and prevalence of sarcopenia in chronic kidney disease**

Sarcopenia is an important issue in dialysis-independent CKD and ESKD patients because it increases the risk of death, cardiovascular complications, the hospitalization rate, and disabilities. Sarcopenia is a strong predictor of mortality in dialysis-independent CKD and hemodialysis (HD) patients (hazard ratio [HR], 2.89; 95% confidence interval [CI], 1.40–5.96; p < 0.004 and HR, 6.99; 95% CI, 1.84–26.58; p = 0.004, respectively) [4,5]. Sarcopenia is a risk factor for cardiovascular events in HD patients (HR, 4.33; 95% CI, 1.51–12.43; p = 0.006) [5]. Sarcopenia is associated with a higher risk of hospitalization in HD patients (relative risk, 2.07; 95% CI, 1.48–2.88; p < 0.001) [6]. Sarcopenic patients exhibited a higher risk of falls than nonsarcopenic patients [7].

Physical inactivity due to sarcopenia results in poor prognosis in CKD patients. Several reports have shown a significant association of physical inactivity with kidney survival and mortality [8,9]. Therefore, maintaining physical performance to prevent sarcopenia is a critical factor in improving the prognosis of CKD patients. The prevalence and incidence of ESKD in Korea are increasing, with >100,000 and 18,642 patients at the end of 2019, respectively [10].

<table>
<thead>
<tr>
<th>Table 1. European and Asian criteria for sarcopenia</th>
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<tbody>
<tr>
<td><strong>European Working Group on Sarcopenia in older people 2 (EWGSOP2)</strong></td>
</tr>
<tr>
<td>(Case finding)</td>
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<tr>
<td>HGS</td>
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<tr>
<td>Chair stand</td>
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<tr>
<td>ASM</td>
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<tr>
<td>ASM/height2</td>
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</tbody>
</table>
| ASM, appendicular skeletal muscle mass; BIA, bioimpedance analysis; DEXA, dual-energy X-ray absorptiometry; HGS, handgrip strength; SARC-F, screening tool for sarcopenia, questionnaire consisting of 5 questions: strength (S), assistance walking (A), rising from a chair (R), climbing stairs (C), and falls (F) on a scale of 0 to 2; SARC-CalF, SARC-F + calf circumference; SPPB, short physical performance battery; TUG, timed up and go.
Therefore, the prevention and treatment of sarcopenia in CKD patients are highly important. Altogether, sarcopenia is a major public health problem and will be even more important in the future given the aging society. The prevalence of sarcopenia in Korean older adults (mean age of 70.3 years) was 32.5% (total), 15.1% (male), and 42.1% (female) based on the Asian Working Group for Sarcopenia (AWGS) 2019 [11].

The prevalence of sarcopenia increases with CKD stage: 17% in stage 3a, 20% in stage 3b, 29% in stage 4, and 38% in stage 5 [12]. A lower lean mass and appendicular skeletal muscle mass index (ASMI) are correlated with glomerular filtration rate (GFR) decline, and a 1 mL/min/1.73 m² decrease in GFR was associated with a 0.03 ± 0.01 kg/m² decrease in the ASMI [13].

The prevalence of sarcopenia ranges from 4% to 42% according to the definition used, the population studied, and the stage of CKD [4,13–17]. A sarcopenia prevalence of 5.9% to 14% was found in dialysis-independent CKD patients [4,13], and that of peritoneal dialysis (PD) patients was from 4% to 15.5% based on the European Working Group for Sarcopenia criteria [15,16]. It was from 1.9% to 40% based on AWGS cutoffs in PD and HD patients [14,17].

The issue is the application of these criteria in clinical practice and the lack of recommended cutoff points in specific populations, such as CKD and ESKD patients. CKD is a condition associated with muscle loss [1,18–20]. There is a possibility of underestimating the clinical significance of sarcopenia in CKD patients [21].

### Risk factors of uremic sarcopenia

Several risk factors for sarcopenia are alleged in CKD and ESKD patients (Fig. 1).

Aging is associated with sarcopenia and an increased prevalence of CKD, which accelerates normal physiological muscle wasting [22]. CKD is associated with chronic low-grade inflammation leading to progressive weight loss, muscle weakness, and disabilities. Inflammatory cytokines, oxidative stress, and inactivity-mediated destruction of protein homeostasis result in the catabolic destruction of structural and functional proteins, resulting in skeletal muscle wasting and a decrease in exercise capacity [23,24].

Inflammation status, i.e., a high malnutrition-inflammation score (MIS) and high levels of high-sensitive C-reactive protein (CRP), interleukin (IL) 6, β2-microglobulin,

---

**Figure 1. Possible etiologies of uremic sarcopenia.**

ATP, adenosine triphosphate; BMI, body mass index; IGF, insulin-like growth factor; IL-6, interleukin 6; IL-1β, interleukin-1β; INF-γ, interferon-γ; MIS, malnutrition-inflammation score; PTH, parathyroid hormone; SGA, subjective global assessment; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha.
and IL-4 is associated with sarcopenia in CKD patients [5,14,25–28].

Older age, male sex, low body mass index (BMI), and longer vintage of dialysis are associated with sarcopenia [12,17,29,30]. Malnutrition, i.e., low albumin/prealbumin levels, a high MIS, and poor nutritional status based on subjective global assessment are also associated with sarcopenia [5,14,17,25,27,31,32]. Low serum vitamin D levels and diabetes mellitus are risk factors for sarcopenia [5,12,17,32]. Depression (odds ratio [OR], 6.87; 95% CI, 2.06–22.96; p = 0.002) and mild cognitive impairment (OR, 6.36; 95% CI, 1.62–34.96; p = 0.008) are associated with sarcopenia [25,27].

Impaired perfusion due to chronic heart failure, decreased oxygen supply to skeletal muscles due to anemia, and decreased skeletal muscle capillaries are other risk factors for sarcopenia [33]. Insulin resistance-associated skeletal muscle atrophy and changes in muscle composition, including fat accumulation, are risk factors for sarcopenia in CKD [33,34]. Inactivity and renin-angiotensin system activation are also mechanisms of exercise intolerance in CKD [33].

Mitochondrial dysfunction of skeletal muscle in CKD is considered to be a cause of loss of muscle mass and exercise capacity [35–38]. CKD patients have decreased muscle mitochondrial content and oxidative capacity along with suppressed activity of various mitochondrial enzymes, leading to impaired energy production. These changes can be found from the early stages of CKD and are aggravated along with CKD progression [37].

Metabolic acidosis can induce glucocorticoid secretion and insulin resistance and increase branched-chain amino acid oxidation in skeletal muscles, which is associated with decreased muscle protein synthesis [39–41]. Acidosis also induces reprogramming of cellular metabolism to mitigate oxidative stress, although this originates from cancer cells [42].

**Gut microbiome and uremic metabolite**

Influx of urea and other retained toxins exerts a change in the gut microbiome in CKD, favoring pathobiont overgrowth. The abundance of bacteria that contain tryptophanase and urease and produce uremic toxins such as indoxyl sulfate (IS), p-cresyl sulfate (PCS), and trimethylamine-N-oxide (TMAO), increases, while the abundance of beneficial bacteria producing short-chain fatty acids, an essential nutrient for the colonic epithelium, decreases [43]. *Lactobacillus* and *Bifidobacterium* are decreased, while Proteobacteria, *Enterobacter*, *Escherichia coli*, *Acinetobacter*, *Clostridium perfringens*, and *Proteus* species are increased in the colon of CKD patients [44].

Disruption of the colonic epithelial tight junction associated with urea influx, increased ammonium production, and decreased epithelial survival results in the loss of integrity and increased intestinal permeability, which allows the translocation of bacteria and lipopolysaccharide (LPS). LPS can activate immune cells through the toll-like receptor 4 (TLR-4) dependent and nuclear factor kappa-B (NF-kB) pathway. And pathobionts can stimulate dendritic cells and produce inflammatory cytokines [45]. Prolonged colonic transit time, dietary restriction of fiber, and metabolic acidosis also directly and indirectly contribute to dysbiosis and the altered intestinal environment [46].

**Role of the gut microbiome and uremic metabolite on the maintenance of skeletal muscle mass**

Reduced muscle mass, altered muscle composition, i.e., increased deposition of lipids or adipocytes within and/or between muscles, and poor physical function are prevalent in ESKD patients. ESKD patients have elevated uremic metabolite levels combined with an altered gut microbiome, increased gut-derived uremic metabolites, and increased epithelial permeability. In turn, the fecal levels of short-chain fatty acids are reduced. The accumulation of gut-derived uremic metabolites within skeletal muscle results in decreased muscle mass or increased lipid content [47]. Increased urea due to decreased kidney function, gut dysbiosis, increased circulating uremic metabolites, and the resultant reduced muscle mass, altered muscle composition, and poor physical function is called the kidney-gut-muscle axis (Fig. 2).

Protein-derived tryptophan is metabolized into indole, which is then absorbed into the bloodstream and oxidized into IS in the liver [48]. Uremic toxins enter target cells via specific transporters, such as the organic anion transporter (OAT), and then, exert their toxicity via the activation of cellular nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, which results in the overpro-
duction of reactive oxygen species (ROS) and inflammatory cytokines [49,50]. IS also enters various cells via OATs (OAT1 and OAT3), and OATs are expressed in muscles [51–53].

IS and PCS accumulate in various organs, including skeletal muscle, in the CKD mouse model [54]. IS exposure inhibited cell proliferation and cell viability and decreased mitochondrial function in mouse muscle cells. CKD patients also showed a significant inverse association between plasma IS levels and skeletal muscle mass [55]. High IS levels were associated with longer HD vintage, and IS was negatively correlated with the skeletal muscle mass index in HD patients. HD patients with high IS levels showed greater loss of hand grip strength (HGS) [56].

Accumulated IS in muscle cells via OAT activates NADPH oxidase and the aryl hydrocarbon receptor (AHR) pathway to cause increased ROS production. Enhanced ROS trigger inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-6, and transforming growth factor beta 1 (TGF-β1), and induce myostatin (negative regulator of muscle growth) and atrogin-1 (muscle-specific ubiquitin ligase family) expression, which are involved in muscle wasting [52].

IS also induces mitochondrial network disintegration through metabolic alterations, such as an increase in antioxidative responses (pentose phosphate pathway and glutathione metabolism), and a decrease in energy generation-related pathways (tricarboxylic acid cycle, glutamine metabolism, and mitochondrial oxidative phosphorylation) in muscle cells, which results in reduced adenosine triphosphate (ATP) production [55]. Muscle mitochondrial dysfunction was found from the early stage of CKD and muscle atrophy followed by exercise impairment [37]. Muscular mitochondria were decreased in the early stage of CKD in mice.

In addition, IS downregulated Klotho expression through ROS-associated NF-κB activation [57]. Klotho levels and skeletal muscle physiology are closely related [58,59]. Klotho-deficient mice showed significantly decreased body weight and forelimb grip strength, and Klotho overexpression restored grip strength and running capacity [60]. It is possible that downregulation of Klotho by IS in muscles is one of the mechanisms of sarcopenia in CKD. IS also has direct toxic effects on myoblasts by decreasing their viability and increasing cell apoptosis [61].

A systematic review and meta-analysis showed that biotic supplements, AST-120, hemodiafiltration, and preservation of residual renal function significantly reduced uremic toxins, including IS and PCS [62]. A reduction in the
elevated AHR transactivating activity by dialysis was also reported [63].

**Strategies to prevent and treat sarcopenia**

Researchers can acquire an impression regarding the treatment strategies based on all of the risk factors mentioned above. Many drugs have been developed to prevent or treat sarcopenia. However, there are no drugs approved by the U.S. Food and Drug Administration for the treatment of sarcopenia [64]. Only a few of them, i.e., Bimagrumab from Novartis and Sarconeos from Biophytis SAS, have recently shown favorable results.

Bimagrumab (BYM338) is a fully human monoclonal antibody, which was developed to treat pathological muscle loss and weakness. It binds to and inhibits activin receptor type-2B and prevents the actions of natural ligands that negatively regulate skeletal muscle growth [65,66]. Activin receptor type-2B blockade with Bimagrumab resulted in the significant loss of fat mass, gain in lean mass, and metabolic improvements during 48 weeks in patients with overweight or obesity who had type 2 diabetes in a phase 2 trial [66].

20-Hydroxyecdysone (20E) is a polyhydroxylated plant steroid that has pharmacological effects in aging and sarcopenia. Sarconeos (BIO101) is a 20E purified investigational drug (≥97%). It targets proto-oncogene protein-c-MAS-1, MAS receptor. BIO101 showed a good safety and pharmacokinetic profile in a phase 1 study for healthy young and older adults [67]. In a phase 2 study (NCT03452488), BIO101 was orally administered for 26 weeks to community-dwelling men and women aged ≥65 years, suffering from age-related sarcopenia (including sarcopenic obesity), and at risk of mobility disability. It was a double-blind, placebo-controlled clinical trial. BIO101 at the highest dose (350 mg, twice daily) showed a clinically meaningful improvement in the 400-m walk test [68].

The main interventions for sarcopenia in CKD patients are aerobic and resistance exercises along with nutritional interventions. Furthermore, optimizing dialysis, vitamin D status, acidosis, and the management of comorbidities such as depression are mandatory [69].

**Megestrol acetate**

HD patients with no residual kidney function (age of 40–80 years) treated with a daily dose of 160 mg of megestrol acetate for 2 to 12 months, experienced an increase in weight (2–9 kg) associated with an increase in the protein catabolic rate, albumin concentration, serum creatinine and urea [70]. In addition, megestrol improved body water distribution with an increase in the intracellular water component and muscle mass.

A review of small retrospective studies showed an increase in BMI, albumin levels, and protein-energy intake after megestrol acetate [71]. A systematic review, especially of dialysis patients, showed a significant increase in body weight, albumin levels, and appetite, although they were small studies (n = 9–32) with a short duration (8–24 weeks) and a high degree of bias. Megestrol acetate was also associated with overhydration, excessive fluid gain, diarrhea, hyperglycemia, suppressed cortisol levels, thrombophlebitis, nausea, and vomiting [72].

**Hormonal therapies**

Nandrolone (decanoate), an androgen and anabolic steroid medication, which is used in the treatment of wasting syndrome and is given through intramuscular or subcutaneous way, injection in HD patients for 6 months significantly increased lean body mass, improved functional capacity (walking and stair-climbing test), and increased appendicular lean mass in a dose-responsive manner [73,74]. Nandrolone combined with intradialytic resistance training for 12 weeks increased the quadriceps muscle cross-sectional area in an additive manner [75].

Oxymetholone, an oral androgen and anabolic steroid, which is also used in the treatment of wasting syndrome and promotion of weight gain and muscle growth, has a higher anabolic effect and lower androgenic activity than testosterone. HD patients showed an increase in fat-free mass, HGS, and physical functioning scores as well as an increase in mRNA expression for myosin heavy chain, and insulin-like growth factor (IGF)-I/II in muscles after oxymetholone administration for 24 weeks [76].

Recombinant human growth hormone (rhGH) increased protein synthesis and decreased protein catabolism in HD and PD patients [77–80]. rhGH increased lean body mass and improved QoL [81]. rhGH also increased HGS after 6 months in elderly HD patients [82]. However, another large randomized controlled trial (RCT) study (OPPORTUNITY)
failed to show an increase in albumin levels, lean body mass, physical capacity, or QoL [83]. rhGH also showed adverse effects, including soft tissue edema, arthralgia, carpal tunnel syndrome, gynecomastia, and dysglycemia [83].

**Vitamin D**

CKD patients in stages 3 to 4 and PD patients with vitamin D deficiency became vitamin D sufficient after vitamin D replacement. They showed improvements in physical function (timed up and go, gait speed, timed chair stand, stair climb, static and dynamic balance) and isometric strength [84]. Vitamin D supplementation in male HD patients also improved muscle mass [85].

However, supplementation of cholecalciferol 50,000 units/week with the target of 25(OH)D concentration >80 nmol/L in HD patients with an initial 25(OH)D concentration <50 nmol/L had no effect on muscle strength or symptoms [86]. Treatment with 50,000 units of oral cholecalciferol supplementation, once weekly for 8 weeks and then monthly for 4 months, to HD patients with a 25(OH)D concentration <60 nmol/L showed higher 25(OH)D and 1,25(OH)2D levels without increased calcium or phosphorus levels. However, muscle strength, functional capacity, and QoL did not change [87].

**Correction of acidosis**

Advanced CKD patients (eGFR of 15–30 mL/min/1.73 m²) with metabolic acidosis presented an increase in the mid-arm circumference, protein intake, and serum albumin levels after oral sodium bicarbonate administration for 2 years [88]. An RCT of PD patients showed an increased body weight and mid-arm circumference and decreased hospitalization after 1 year of high alkali dialysate [89]. In addition, a high bicarbonate dialysate concentration of 40 mmol/L resulted in better control of acidosis and increased triceps skin fold thickness compared to a bicarbonate concentration of 30 mmol/L [90].

**Biotics**

Because gut dysbiosis is associated with systemic inflammation, the administration of biotics can be an indirect target to ameliorate muscle wasting. *Lactobacillus* species and *Bifidobacterium* ameliorated muscle wasting in mouse models, and *Lactobacillus* exhibited the potential to restore microbiome balance, and the gut permeability, which was associated with the improvement in muscle mass or strength, was reduced [91]. However, probiotics containing *Lactobacillus* and *Bifidobacterium* did not show meaningful effects on muscle mass and function in humans, because of the scarcity of studies, the variability in the populations, and the difficulty in accurately and reproducibly measuring muscle mass and function [92].

Nonetheless, *Bifidobacterium longum* was significantly increased after prebiotic administration in super-elderly patients with sarcopenia. The skeletal muscle mass index was increased, while the body fat percentage was decreased [93]. A randomized, double-blind, placebo-controlled, crossover trial using synbiotics over 6 weeks in predialysis CKD patients showed an increase in *Bifidobacterium* and *Lactobacillus* after synbiotic therapy as well as a decrease in Clostridiales. The *Bifidobacterium* change showed an inverse correlation with the concentration of serum IS and PCS [94].

Although there is no data about the effect of biotics on muscle mass, muscle strength, or physical function, there are several reports about the relationship between biotics and surrogate outcomes such as inflammation, oxidative stress, uremic toxins, and endotoxins.

Probiotic supplementation in HD patients reduced systemic inflammatory responses, which was associated with an increase in Tregs and a decrease in proinflammatory monocytes [95]. Probiotic, prebiotic, and synbiotic administration also reduced CRP, IL-6, and IS levels, and increased high-density lipoprotein cholesterol in dialysis patients [96]. Synbiotics were more effective than probiotics in improving inflammatory markers, endotoxins, and anti-heat shock protein70 serum levels in HD patients [97]. Synbiotics also reduced serum IS, PCS, and urea in HD patients [98].

**Oral adsorbents**

AST-120 adsorbs uremic toxins and precursors, including indole, and excretes them into the feces.

AST-120 administration increased *Lactobacillus* in CKD rats. The microbiota composition after AST-120 treatment was between that of normal and CKD rats [99]. CKD rats
<table>
<thead>
<tr>
<th>Drug</th>
<th>Patient</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megestrol acetate [49–51]</td>
<td>HD patients</td>
<td>↑ Appetite, ↑ weight/BMI, ↑ protein catabolic rate, ↑ serum albumin/creatinine/urea, ↑ intracellular water component, ↑ muscle mass</td>
</tr>
<tr>
<td>Nandrolone [52–54]</td>
<td>HD patients</td>
<td>↑ lean body mass, ↑ walking speed, stair-climbing, ↑ appendicular lean mass, ↑ quadriceps muscle cross-sectional area (+resistance training)</td>
</tr>
<tr>
<td>Oxymetholone [55]</td>
<td>HD patients</td>
<td>↑ lean body mass, HGS, physical functioning scores, ↑ mRNA expression for myosin heavy chain, ↑ IGF-I/II</td>
</tr>
<tr>
<td>Recombinant human growth</td>
<td>HD and PD patients</td>
<td>↑ protein synthesis, ↓ protein catabolism, ↑ lean body mass, HGS, ↑ QoL</td>
</tr>
<tr>
<td>Vitamin D [63, 64]</td>
<td>CKD stage 3–4 and PD patients</td>
<td>↑ vitamin D concentration</td>
</tr>
<tr>
<td>Prebiotics [93]</td>
<td>Super-elderly with sarcopenia</td>
<td>↑ <em>Bifidobacterium longum</em>, ↑ skeletal muscle index, ↓ body fat percentage</td>
</tr>
<tr>
<td>Synbiotics [94]</td>
<td>Predialysis CKD patients</td>
<td>↑ <em>Bifidobacterium</em>, Lactobacillus, Clostridium, ↑ serum indoxyl sulfate, p-cresyl sulfate</td>
</tr>
<tr>
<td>Probiotics [95]</td>
<td>HD patients</td>
<td>↓ system inflammation</td>
</tr>
<tr>
<td>Pre-, pro-, synbiotics [96]</td>
<td>Dialysis patients</td>
<td>↓ C-reactive protein, interleukin-6, indoxyl sulfate, ↑ high-density lipoprotein</td>
</tr>
<tr>
<td>Synbiotics [97, 98]</td>
<td>HD patients</td>
<td>↓ inflammation marker, endotoxin, anti-heat shock protein 70, ↑ indoxyl sulfate, p-cresyl sulfate, urea</td>
</tr>
<tr>
<td>AST-120 [103]</td>
<td>Advanced CKD patients with serum</td>
<td>↑ gait speed, ↑ body pain, vitality, symptoms/problems, cognitive function scores, ↓ HbA1c, ↑ QUICK</td>
</tr>
<tr>
<td>Bimagrumab (BYM338) [45]</td>
<td>Overweight or obese type 2 diabetes</td>
<td>↓ fat mass, ↑ lean mass, ↓ HbA1c, ↑ QUICK, → HOMA2 (36-week), → Matsuda index (48-week)</td>
</tr>
<tr>
<td>Sarconeos (BIO101) [47]</td>
<td>Community-dwelling men and women aged ≥ 65 years, age-related sarcopenia, at risk of disability</td>
<td>↑ 400-m walk test</td>
</tr>
</tbody>
</table>

BMI, body mass index; CKD, chronic kidney disease; HbA1c, hemoglobin A1c; HD, hemodialysis; HGS, handgrip strength; HOMA, homeostatic model assessment; IGF, insulin-like growth factor; mRNA, messenger RNA; PD, peritoneal dialysis; QoL, quality of life; QUICK, quantitative insulin sensitivity check index; TUG, timed up and go.

Bimagrumab, Novartis; Sarconeos, Biophytis SAS.
showed elevated plasma endotoxin, IL-6, TNF-α, monocyte chemoattractant protein-1 (MCP-1), cytokine-induced neutrophil chemoattractant-3 (CINC-3), L-selectin, intercellular adhesion molecule-1 (ICAM-1), and malondialdehyde levels and depletion of the colonic epithelial tight junction proteins, claudin-1, occludin, and zonular occludens-1 (ZO-1). AST-120 showed partial restoration of epithelial tight junction proteins and a reduction in plasma endotoxin and markers of oxidative stress and inflammation [100].

AST-120 prevented the tissue accumulation of IS and PCS in skeletal muscle, which resulted in the amelioration of muscle atrophy (cross-sectional area) [54]. AST-120 prevented CKD-induced physical inactivity mainly by maintaining mitochondrial function, suppressing atrogin-1/myostatin expression, and recovering Akt phosphorylation in skeletal muscle [101]. A reduction in oxidative stress via AST-120 improved exercise capacity and mitochondrial biogenesis of skeletal muscle [33,101]. AST-120 significantly restored skeletal muscle weight in CKD mice. In addition, AST-120 increased the renal expression of Klotho, which was decreased under uremic conditions [102]. Klotho levels and skeletal muscle physiology were closely related, as we mentioned above [58,59].

In a human RCT, gait speed significantly increased over 1 year after carbonaceous oral adsorbent administration. Bodily pain, vitality, symptoms/problems, and cognitive function also improved in the oral adsorbent group, while the quality of social interactions and kidney disease effects decreased in the control group [103]. Prospective studies with longer follow-up durations, larger sample sizes, and interventions to lower uremic toxins are necessary to elucidate the role of uremic toxins in CKD patients with sarcopenia.

The effectiveness of pharmacologic interventions mentioned above is summarized in [Fig. 3].

In conclusion, aerobic and resistance exercise, along with nutritional interventions, including oral and intradialytic parenteral nutritional supplements, has been the main preventive or treatment strategies for sarcopenia in dialysis-independent CKD and ESKD patients. In addition, anabolic hormones, including anabolic steroids and growth hormones, and appetite stimulants showed beneficial effects on biochemical markers such as serum albumin level, both muscle strength and mass, and physical function. Correction of metabolic acidosis and vitamin D status also showed favorable effects on physical function, muscle mass, and strength. Biotics improved the gut microbiome composition and the level of uremic toxins, endotoxins, inflammation, and oxidative stress. Oral adsorbents also showed beneficial effects on the restoration of the gut microbiome composition and intestinal wall integrity as well as on the skeletal muscle physiology. A multifaceted approach can be helpful to lessen the burden of sarcopenia in CKD patients (Fig. 3).

Conflicts of interest
The author has no conflicts of interest to declare.

Data sharing statement
The data presented in this study are available on request from the corresponding author.

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Validation of operational definitions of mortality in a nationwide hemodialysis population using the Health Insurance Review and Assessment Service databases of Korea

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Background: Health Insurance Review and Assessment Service’s (HIRA) claims data have been used in studies of hemodialysis patients even though information about mortality is not provided in this database. Mortality analysis using HIRA data has been conducted using various operational definitions that have not been validated. This study aimed to validate operational definitions of mortality for maintenance hemodialysis patients that have been used when analyzing the Korean HIRA database.

Methods: This study utilized claims data of the Korean National Health Insurance Service (NHIS) between January 2008 and December 2019. We estimated mortality based on operational definitions applied in previous studies using the HIRA database and compared it with NHIS mortality information to validate accuracy.

Results: A total of 128,876 patients who started maintenance hemodialysis between January 2009 and December 2019 were analyzed. The accuracy of estimated mortality was the highest at 96% in the group where mortality was defined as an absence of claims data for 150 days. If the period of no claims data was set to 90 days or less, there was a risk of overestimating the mortality for the entire study period. When it was set to 180 days or more, there was a risk of underestimating the mortality, as the follow-up time was close to the end of the study period.

Conclusion: When mortality analysis of maintenance hemodialysis patients is performed using HIRA data, it is most accurate to set the operational definition period as the absence of claims data for 150 days.

Keywords: Dialysis, Health insurance, Korea, Mortality
Introduction

National population-based studies using data from national health insurance systems can facilitate long-term tracking of the healthcare environment and the collected data can be used for numerous studies including outcome studies, epidemiological studies, and pharmacovigilance studies [1–3]. Korea has a universal, single-payer national health insurance system where the entire Korean population and healthcare providers are eligible to register for the national health insurance program comprising the National Health Insurance Service (NHIS) and Medical Aid [4,5]. In particular, the specific code (V001) assigned to hemodialysis (HD) patients is a useful tool to identify patients on maintenance HD in Korea, and several studies on this population have been conducted [6,7]. All medical claims for medical services issued by healthcare providers are reviewed and assessed by the Health Insurance Review and Assessment Service (HIRA), and the medical expenses are reimbursed by the National Health Insurance Cooperation (NHIC) [8]. Therefore, all medical claims data for medical services provided for dialysis patients enrolled in the national health insurance program or Medical Aid is stored and managed in the HIRA and NHIS databases. Using NHIS or HIRA databases, it is possible to identify every end-stage kidney disease (ESKD) patient within the entire Korean population and analyze the claims data of all ESKD patients on dialysis therapy.

Nevertheless, the HIRA database does not provide detailed information on mortality, whereas it can be obtained from the NHIS database. All-cause mortality is the most objectively measurable surrogate endpoint because it has minimal chance of being affected by bias in patient selection, missing data, or misclassification of the cause of death [9]. Although it is ideal to merge NHIS data with that from Statistics Korea to investigate the time of death as well as the cause of death [10–12], analysis of all-cause mortality is feasible based on mortality information from the NHIS database. In the Korean HD population, alternatively, studies on all-cause mortality have been conducted using HIRA data by defining all-cause mortality as death where no claims were made for a certain period of time such as 30 days, 90 days, or more than 180 days [13–16].

These operational definitions of mortality have been accepted based on the consensus that chronic dialysis patients would have died without the use of medical services for several months. However, this has yet to be verified. This study aimed to validate the operational definition period for mortality that has been used in the analyses of claims data in the HIRA database.

Methods

Data sources

This study utilized claims data in the NHIS database from January 2008 to December 2019. We collected demographic information, diagnostic codes based on the International Classification of Diseases, Revision 10 (ICD-10), procedure codes, and data on mortality of patients on maintenance HD. This study was approved by the Institutional Review Board (IRB) of Asan Medical Center (No. 2020-0576) and complied with the principles of the Declaration of Helsinki. Informed consent was waived by the IRB due to the anonymized and retrospective design of the study.

Data population

We defined ESKD patients as those with a diagnosis of chronic kidney disease (diagnosis code: N18 or N19) who underwent HD treatment (procedure code: O7020 or O9991) or those patients with the specific code of V001, indicating HD patients. We extracted claims data of maintenance HD patients who had HD treatment over 90 consecutive days between January 2008 and December 2019.

Study population

To identify incident HD patients aged over 19 years, we excluded patients less than 19 years old and patients who had a specific code for HD (V001) or procedure codes for HD (O7020, O9991) more than 90 days during the year before the initiation of HD treatment. Among them, we identified patients who started HD during the study period and maintained HD for at least 90 days. Patients who underwent kidney transplantation (KT) or peritoneal dialysis (PD) in the study period were also excluded. Therefore, we analyzed a total of 128,876 HD patients who continued HD over 90 days and remained on chronic HD till the end of the study period. Deceased patients, who were confirmed
to be deceased during the study period based on analysis of the Certificate Database of NHIS, were assigned to group A. Deceased patients defined according to the period of absence of any claims data for medical service use were assigned to group B. A flow diagram for the selection of the study population is provided in Fig. 1.

**Definitions**

Patients on maintenance HD were defined as ESKD patients who underwent HD over 90 days. Mortality in group A was defined as patient death as identified in the NHIS database. In group B, mortality by operational definitions was defined as the absence of any claims data for medical service use over 30, 60, 90, 120, 150, and 180 days. Namely, groups B30, B60, B90, B120, B150, B180 comprised patients with presumed deaths defined by operational definition as the absence of any claims data for medical service use over 30, 60, 90, 120, 150, and 180 days, respectively. Mortality date by operational definition was defined as the first date of the period of the absence of any medical claims data for medical service use. If mortality was not identified at the end of the study period, December 2019, it was regarded as censored data. Patients who underwent KT or PD were defined as those who had codes related to KT (Z940, R3280) or procedure codes for PD (O7061, O7062, O7071, O7072) during the study period.

**Statistical analysis**

Demographic data are presented as frequencies in the case of categorical variables or as means and standard deviations or medians and interquartile ranges (IQRs) in the case of continuous variables. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated according to a 2 × 2 contingency table (Table 1).

Patients’ survival probabilities were assessed by Kaplan-Meier survival analysis. Statistical analyses were performed using SAS Enterprise Guide version 7.1 (SAS Institute).

**Results**

There were 151,382 (58.4% male) incident dialysis patients on maintenance HD between January 2009 and December 2019. Among these, 128,876 patients (58.0% male) had not undergone PD or KT. Mean follow-up period was 3.26 ± 2.8 years (median, 2.61 years; IQR, 0.97–4.93 years) and mean age was 66.09 ± 13.8 years. Among the total 128,876 patients, 66,520 deaths were identified in the NHIS data during the study period; these cases were assigned to group A. The all-cause mortality of groups during the study period is presented in Table 2. The total mortality of group B30 during the study period was 89,277, which was the largest difference compared with that of group A (n = 66,520), whilst the smallest difference was noted in group B150 (n = 66,514).

Sensitivity, specificity, PPV, NPV, and overall accuracy were calculated by comparing mortality between the A and B groups (Table 3). In groups B120, B150, and B180, accuracy was more than 95%, with group B150 showing the
Table 1. Two-by-two contingency table regarding the agreement in death classification between the NHIS and no insurance claims data for a specific period

<table>
<thead>
<tr>
<th>No insurance claims data for a specific period</th>
<th>Certificate database of NHIS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>Death</td>
<td>Survival</td>
</tr>
<tr>
<td>Death</td>
<td>TP</td>
<td>FN</td>
</tr>
<tr>
<td>Survival</td>
<td>FP</td>
<td>TN</td>
</tr>
<tr>
<td>Total</td>
<td>TP + FN</td>
<td>FP + TN</td>
</tr>
</tbody>
</table>

Sensitivity = TP/(TP + FN); specificity = TN/(FP + TN); PPV = TP/(TP + FP); NPV = TN/(FN + TN); overall accuracy = (TP + TN)/(TP + FP + FN + TN).

NHIS, National Health Insurance Service; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

Table 2. Mortality of incident patients on maintenance hemodialysis according to group classification

<table>
<thead>
<tr>
<th>Duration (yr)</th>
<th>No. of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>0.5</td>
<td>24,789</td>
</tr>
<tr>
<td>1.0</td>
<td>7,777</td>
</tr>
<tr>
<td>1.5</td>
<td>5,572</td>
</tr>
<tr>
<td>2.0</td>
<td>4,429</td>
</tr>
<tr>
<td>2.5</td>
<td>3,749</td>
</tr>
<tr>
<td>3.5</td>
<td>2,829</td>
</tr>
<tr>
<td>4.0</td>
<td>2,421</td>
</tr>
<tr>
<td>4.5</td>
<td>2,079</td>
</tr>
<tr>
<td>5.0</td>
<td>1,838</td>
</tr>
<tr>
<td>5.5</td>
<td>1,585</td>
</tr>
<tr>
<td>6.0</td>
<td>1,294</td>
</tr>
<tr>
<td>6.5</td>
<td>1,058</td>
</tr>
<tr>
<td>7.0</td>
<td>925</td>
</tr>
<tr>
<td>7.5</td>
<td>766</td>
</tr>
<tr>
<td>8.0</td>
<td>605</td>
</tr>
<tr>
<td>8.5</td>
<td>482</td>
</tr>
<tr>
<td>9.0</td>
<td>422</td>
</tr>
<tr>
<td>9.5</td>
<td>277</td>
</tr>
<tr>
<td>10.0</td>
<td>207</td>
</tr>
<tr>
<td>10.5</td>
<td>94</td>
</tr>
<tr>
<td>11.0</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>66,520</td>
</tr>
</tbody>
</table>

Group A is defined as patients identified as deceased in the NHIS database. Groups B30, B60, B90, B120, B150, and B180 are patient groups with presumed deaths defined by operational definition as the absence of any claims data for medical service use over 30, 60, 90, 120, 150, and 180 days, respectively.

We performed survival analysis based on Kaplan-Meier analysis; survival curves are depicted in Fig. 2. Mortality was overestimated in groups B30 and B60 compared to the registration information present in the NHIS data (group A). Group B90 had a greater risk of mortality rate overestimation than groups B120, B150, B180 as well as the lowest overall accuracy (Fig. 3). Compared to the mortality dates for group A, the mortality dates of groups B120, B150, and B180 were identical to those of group A in 88.3%, 88.5%, and 88.6% of deceased patients, respectively. Median difference in mortality date between group A and groups B120 (IQR, 1–14), B150 (IQR, 1–13), and B180 (IQR, 1–12) was 3 days.
Table 3. Sensitivity, specificity, PPV, NPV, and accuracy of groups defined based on operational definitions of mortality

<table>
<thead>
<tr>
<th>Group</th>
<th>% (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>B30</td>
<td>99.5 (99.4–99.5)</td>
<td>63.0 (62.6–63.4)</td>
<td>74.2 (73.9–74.4)</td>
<td>99.1 (99.0–99.2)</td>
<td>81.8 (81.6–82.0)</td>
<td></td>
</tr>
<tr>
<td>B60</td>
<td>98.5 (98.4–98.6)</td>
<td>84.3 (84.0–84.5)</td>
<td>90.0 (89.7–90.3)</td>
<td>98.1 (98.0–98.2)</td>
<td>91.6 (91.4–91.7)</td>
<td></td>
</tr>
<tr>
<td>B90</td>
<td>97.6 (97.5–97.7)</td>
<td>91.5 (91.3–91.7)</td>
<td>92.4 (92.2–92.6)</td>
<td>97.3 (97.2–97.4)</td>
<td>94.7 (94.5–94.8)</td>
<td></td>
</tr>
<tr>
<td>B120</td>
<td>96.9 (96.7–97.0)</td>
<td>94.9 (94.8–95.1)</td>
<td>95.3 (95.2–95.5)</td>
<td>96.6 (96.4–96.7)</td>
<td>95.9 (95.8–96.0)</td>
<td></td>
</tr>
<tr>
<td>B150</td>
<td>96.1 (96.0–96.0)</td>
<td>95.9 (95.7–96.0)</td>
<td>96.2 (96.0–96.3)</td>
<td>95.9 (95.7–96.0)</td>
<td>96.0 (95.9–96.1)</td>
<td></td>
</tr>
<tr>
<td>B180</td>
<td>95.3 (95.2–95.5)</td>
<td>96.5 (96.3–96.6)</td>
<td>96.7 (96.5–96.8)</td>
<td>95.1 (94.9–95.2)</td>
<td>95.9 (95.8–96.0)</td>
<td></td>
</tr>
</tbody>
</table>

Groups B30, B60, B90, B120, B150, and B180 are patient groups with presumed deaths defined by operational definition as the absence of any claims data for medical service use over 30, 60, 90, 120, 150, and 180 days, respectively. CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Figure 2. Survival curves for all groups. Registration information; the survival curve of group A is the reference. Groups B30, B60, B90, B120, B150, and B180 are patient groups with presumed deaths defined by operational definition as the absence of any claims data for medical service use over 30, 60, 90, 120, 150, and 180 days, respectively.
Figure 3. Comparison of the survival curves of groups based on operational definitions of mortality with that of the group with mortality defined based on registration information (group A). (A) Comparison of the survival curve of group B90 with that of group A. (B) Comparison of the survival curve of group B120 with that of group A. (C) Comparison of the survival curve of group B150 with that of group A. (D) Comparison of the survival curve of group B180 with that of group A. Registration information; the survival curve of group A was set as the reference. Groups B90, B120, B150, and B180 are patient groups with presumed deaths defined by operational definition as the absence of any claims data for medical service use over 90, 120, 150, and 180 days, respectively.

The probabilities of a difference of a week or less between the mortality dates for group A and B patients were 95.7%, 95.9%, and 96.0% for groups B120, B150, and B180, respectively (Table 4).
Discussion

Despite the usefulness of claims data from the health insurance system, analysis of data from NHIS and HIRA databases poses some unique challenges. NHIS data are difficult to access, and HIRA data do not provide detailed mortality information. Given that all-cause mortality is a surrogate endpoint and the hardest objectively measurable outcome, its unavailability in the HIRA data is a disadvantage. To analyze NHIS data, it is necessary to visit one of a limited number of analyzing centers that can only be accessed during the daytime for a limited period, an untenable situation for the majority of physicians in clinical practice. Conversely, the HIRA database provides more favorable accessibility with no daytime access restrictions and can be analyzed in one’s own office via remote access at any time.

To study patients on maintenance HD, nephrologists have applied operational definitions to HIRA data by interpreting the absence of any claims data as death [13–16].

Clinically, it is very hard for ESKD patients on maintenance HD to survive over several months without using any medical services. However, when analyzing HIRA data using an operational definition of death, a patient who is alive may be misclassified as dead in some circumstances, including cases where insurance eligibility was suspended for a period due to long-term overseas stay, emigration, or long-term nonpayment of health insurance premiums. It is not clear how much these exceptional cases affect the agreement between mortality from the NHIS database and presumed mortality from the HIRA database when mortality analysis of HIRA data is performed using operational definitions.

Our study findings indicate that when a period of no claims data of 150 days was used, it provided the most accurate estimate of mortality rate based on NHIS data. The period of 30 days was most sensitive but also least accurate because, in Korea, healthcare centers only report monthly claims data to the HIRA. When the period of no claims data was defined as 90 days or less, the possibility of mortality rate overestimation was high. If mortality is defined by as long as possible a period of no claims data, mortality may be underestimated despite a high PPV. This is because the longer the period of no claims data, the longer the immortal time at the end of the follow-up period. This implies that if the period of no claims data is too long, the patient’s death is reflected with less sensitivity, resulting in low accuracy. This effect was more pronounced as longer the survival duration (Fig. 3). Therefore, when the period of no claims data was set to 90 days or less, there was a high risk of overestimating the mortality rate during the entire study period. Conversely, when it was set to 180 days or more, there was a risk of underestimating the mortality rate.

We investigated the difference between mortality date estimated by each operational definition and that of the NHIS database. In this study, an operational definition of mortality date was considered as the first date of the period that the patient had not used any medical services according to each definition. There was a median difference of 3 days between the date of death in the NHIS data and that of groups B120, B150, and B180 (IQR of 1–14, 1–13, and 1–12, respectively). These results appear to reflect the characteristics of patients on maintenance HD who regularly undergo HD treatment at least every 2 to 3 days.

Mortality dates of groups B120, B150, and B180 were identical to those in the NHIS database in 88.3%, 88.5%, and 88.6% of cases, respectively, and for cases where the mortality date was estimated by the operational definition, there was a difference of a week or less in the mortality date from that of the NHIS in 95.7%, 95.9%, and 96.0% of cases.
respectively. These results imply that judicious establishment of operational definitions can provide reliable outcomes in mortality analysis using Korean HIRA data.

This study had several limitations. First, this study utilized the Korean administrative database and the Korean national health insurance system, and thus cannot be generalized to other nations with different health insurance systems or administrative databases. Second, it is not feasible to investigate the cause of death using HIRA and NHIS databases, hence analysis based on the cause of death still requires merging of data from these databases with cause of death information from Statistics Korea. Last, the best accuracy of the applied operational definitions for all-cause mortality analysis in the present study was 96%; remaining inaccuracies should be further reduced by finding more optimal tools in future investigations.

In conclusion, the operational definition for mortality was most accurate when the period of no claims data was set to 150 days. Thus, absence of claims data for 150 days is an acceptable operational definition for the analysis of all-cause mortality in patients on maintenance HD when using HIRA claims data. Future studies using HIRA data should focus on the interpretation of mortality data and clarify the limitations of analyzing mortality rates obtained by operational definitions.

Conflicts of interest

All authors have no conflicts of interest to declare.

Funding

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Data sharing statement

The data presented in this study are available with permission of the Korean National Health Insurance Service (NHIS).

Authors’ contributions

Conceptualization, Validation: DHL, HSL
Data curation: YJK, HK, HSL
Formal analysis: DHL, YJK, HK
Funding acquisition: HK, HSL
Investigation, Software: DHL, YJK, HSL
Methodology: All authors
Project administration: YJK
Resources: YJK, HK
Supervision, Visualization: HSL
Writing–original draft: All authors
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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Glomerulonephritis following COVID-19 infection or vaccination: a multicenter study in South Korea

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Background: Despite the widespread impact of the severe acute respiratory syndrome coronavirus 2 (coronavirus disease 2019, COVID-19) and vaccination in South Korea, our understanding of kidney diseases following these events remains limited. We aimed to address this gap by investigating the characteristics of glomerular diseases following the COVID-19 infection and vaccination in South Korea.

Methods: Data from multiple centers were used to identify de novo glomerulonephritis (GN) cases with suspected onset following COVID-19 infection or vaccination. Retrospective surveys were used to determine the COVID-19–related histories of patients who were initially not implicated. Bayesian structural time series and autoregressive integrated moving average models were used to determine causality.

Results: Glomerular diseases occurred shortly after the infection or vaccination. The most prevalent postinfection GN was podocytopathy (42.9%), comprising primary focal segmental glomerulosclerosis and minimal change disease, whereas postvaccination GN mainly included immunoglobulin A nephropathy (IgAN; 57.9%) and Henoch-Schönlein purpura nephritis (HSP; 15.8%). No patient progressed to end-stage kidney disease. Among the patients who were initially not implicated, nine patients with IgAN/HSP were recently vaccinated against COVID-19. The proportion of glomerular diseases changed during the pandemic in South Korea, with an increase in acute interstitial nephritis and a decrease in pauci-immune crescentic GN.

Conclusion: This study showed the characteristics of GNs following COVID-19 infection or vaccination in South Korea. Understanding these associations is crucial for developing effective patient management and vaccination strategies. Further investigation is required to fully comprehend COVID-19’s impact on GN.

Keywords: COVID-19, COVID-19 vaccines, Glomerulonephritis, Kidney diseases, Multicenter study
**Introduction**

South Korea was among the early-hit countries during the novel coronavirus severe acute respiratory syndrome coronavirus 2 (coronavirus disease 2019, COVID-19) pandemic, with the first confirmed case reported on January 20, 2020. By December 2022, over 29 million cumulative COVID-19 cases were diagnosed, accounting for 56.3% of the total population, while COVID-19 vaccination doses exceeded 94 million in South Korea [1,2]. Both COVID-19 infection and vaccines have been linked to autoimmune glomerulonephritis (GN) through the activation of innate and/or adaptive immune responses [3,4]. Additionally, COVID-19 is associated with acute kidney injury (AKI) due to excessive cytokine levels and endothelial damage, which are common extrapulmonary complications [3]. The incidence and characteristics of kidney disease following COVID-19 infection or vaccination in South Korea remain unclear, with sporadic reports from single institutions regarding temporal relationships with COVID-19 vaccination [5–8].

Published cases have indicated that COVID-19 and vaccination are predisposing factors for kidney disease [3,4,9–14]. Following COVID-19, AKI has emerged as a common complication, and certain glomerular diseases have also been associated with COVID-19 [3,13,14]. A population-based retrospective study also revealed an increased risk of glomerular disease relapse after receiving COVID-19 vaccines [15]. These findings underscore the importance of recognizing COVID-19 when managing patients with kidney disease. To effectively respond to the ongoing pandemic and future developments in COVID-19 [16], understanding how patients with GNs are affected by COVID-19 events in real-world settings is crucial.

This study comprehensively characterizes kidney diseases following COVID-19 infection or vaccination in South Korea, utilizing data from multiple hospitals. We also investigate the potential impact of COVID-19 on the occurrence of kidney diseases during the pandemic era.

**Methods**

The study protocol was approved by the Institutional Review Board (IRB) of Yonsei University Health System (No. 4–2022–1236). Written consent to publish was waived by the IRB due to the retrospective nature and minimal expected risk in this study.

**Study design**

This study addresses three main issues. We identified all *de novo* GN cases with suspected onset after COVID-19 infection or vaccination from multiple centers (Fig. 1A). Second, we determined the COVID-19–related histories among patients who were initially not implicated by identifying close temporal relationships in a single center (Fig. 1B). Finally, we examined the changes in biopsies during the COVID-19

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**Figure 1. Study flow.** (A) Multicenter and (B) single-center analysis.

COVID-19, coronavirus disease 2019; GN, glomerulonephritis; HSP, Henoch-Schönlein purpura; IgAN, immunoglobulin A nephropathy.

166 www.krcp-ksn.org
vaccination or pandemic era. The histopathological re-
view included the use of light microscopy with periodic
acid-Schiff, trichrome, silver, and hematoxylin and eosin
staining, immunofluorescence microscopy for immuno-
globulins and complements, and electron microscopy. The
biopsies for the transplanted kidney were excluded. The
detailed methods are provided below.

**De novo glomerulonephritis cases after COVID-19 infec-
tion or vaccination: multicenter analysis**

Data were retrospectively collected from multiple centers
(Severance Hospital, Yongin Severance Hospital, Wonju
Severance Christian Hospital, Ilsan Paik Hospital, and Busan
Paik Hospital) between April 2021 and March 2023 us-
ing a de-identified electronic documents (Fig. 1A). Patients
with a clinically suspected association between COVID-19
events and GNs were included. The clinical onset of GNs in
relation to COVID-19 events was evident in collected pa-
tients, most of whom had latent period of no more than 50
days after being infected or vaccinated, although the exact
latent period remains unknown in some patients. Renal
pathology experts thoroughly reviewed all biopsies, and
relevant laboratory findings were extracted from electronic
medical records. Notably, two cases demonstrating tempo-
ral associations with COVID-19 vaccines, lupus nephritis
and pauci-immune crescentic GN, have been reported pre-
viously [8,17].

**Investigation of COVID-19–related history in patients with
biopsy-proven kidney disease in a single center**

This study focused on elucidating the history of COVID-19
vaccination and infection in patients with GN. We iden-
tified COVID-19–related histories among patients who
were initially not suspected of having an association with
COVID-19 events. To assemble this subset of previously
unsuspected biopsies, we excluded those analyzed in the
multicenter series and those taken before April 2021 when
the nationwide vaccination program commenced. There-
fore, we meticulously collected vaccine type, vaccination
date, and infection date data from patients who underwent
native kidney biopsies at Severance Hospital between April
2021 and December 2022 (Fig. 1B). The investigation was
conducted during outpatient visits, and comprehensive
information was gathered by reviewing electronic medical
records.

Concerning patients with included immunoglobulin A
nephropathy (IgAN) or Henoch-Schönlein purpura nephri-
tis (HSP), we conducted an additional investigation into
the time of onset. Utilizing the same timeframe (50 days)
as the multicenter case series, we identified IgAN and HSP
cases that could be temporally linked to vaccination.

**Changes in glomerulonephritis diagnoses during the
COVID-19 pandemic**

We analyzed the changes in GN diagnoses throughout
the prepandemic and pandemic periods (2016–2022) at
Severance Hospital. For statistical purposes, the biopsies
were broadly categorized into 10 groups, including IgAN/
HSP, podocytopathy (comprising minimal change disease
[MCD] and focal segmental glomerulosclerosis [FSGS]),
immune complex-mediated GN (encompassing lupus
nephritis, C3 GN, and membranoproliferative GN), mem-
branous nephropathy, other nephropathies (e.g., diabetes
mellitus nephropathy, hypertensive nephropathy, or para-
protein-related renal disease), pauci-immune crescentic
GN (associated with anti-neutrophil cytoplasm antibodi-
es), acute interstitial nephritis (AIN), acute tubular injury
(ATI), thrombotic microangiopathy (TMA), or nonspecific
histology. Secondary FSGS cases attributed to clear provo-
cative lesions (e.g., hypertensive nephropathy) were clas-
sified accordingly. Biopsies with insufficient glomeruli for
a definitive diagnosis were excluded, and the nonspecific
histology category was applied to cases with near-normal
glomeruli, tubulointerstitium, and blood vessels.

**Statistical analyses**

Continuous variables are expressed as means with stan-
dard deviations (SD) for normally distributed data and me-
dians with interquartile ranges (IQR) for non-normally dis-
buted data, whereas categorical variables are expressed
as numbers and proportions. To evaluate the causal effects
of COVID-19 vaccination and infection on **de novo** GNs, a
Bayesian structural time-series model was performed using
R ‘CausalImpact’ package [18]. The causal effect was de-
termined by assessing the disparity between predicted and
observed outcomes. This evaluation helped establish the
difference in the proportion of newly diagnosed GN cases at Severance Hospital before and after the commencement of the nationwide vaccination program (April 1, 2021), and before and after the peak of the COVID-19 pandemic (February 1, 2022) in South Korea. This approach considered that the nationwide vaccine program began in March 2021, and COVID-19 cases surged significantly as of February 2022 in South Korea, considering weeks of latent period [1,2,4,14]. An additional time-series analysis was conducted using an autoregressive integrated moving average (ARIMA) model. The Box-Cox transformation was performed for non-normally distributed time-series data. The auto.arima function in R ‘forecast’ package was used to automatically select an optimal model [19]. The Ljung-Box Q statistic was used to evaluate the goodness of fit of the automatically selected ARIMA models. The p-value for Ljung-Box Q statistics > 0.05 indicates that the selected ARIMA model fits well and no autocorrelation exists in the residual. Both step and slope changes following the interventions (i.e., COVID-19 vaccination or infection) were examined. All statistical analyses were performed using R (version 4.2.3; R Foundation for Statistical Computing, http://www.r-project.org) with a significance level of p < 0.05.

Results

Clinical characteristics of patients with glomerulonephritis cases following COVID-19 infection

Clinical information from seven patients whose GN was suspected of developing following COVID-19 was collected in a multicenter study. The age at renal biopsy ranged from 24 to 76 years. The pathological diagnoses included ATI (14.3%), TMA with hypertensive nephropathy (14.3%), FSGS, collapsing variant (14.3%), MCD (28.6%), IgAN (14.3%), and HSP (14.3%). Patients with FSGS and IgAN had concurrent ATI. AKI with or without hematuria and proteinuria was the predominant clinical manifestation in three patients (42.9%). These symptoms were observed between 10 and 35 days after infection (Table 1).

Clinical characteristics of patients with glomerulonephritis cases following COVID-19 vaccination

Nineteen patients in whom GN was suspected after

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**Table 1. Clinical Characteristics of Patients from Multicenter with Glomerulonephritis Following COVID-19 Infection**

<table>
<thead>
<tr>
<th>Pathologic diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Underlying diseases</th>
<th>Clinical presentation</th>
<th>Time of biopsy</th>
<th>Treatment</th>
<th>Scr at biopsy (mg/dL)</th>
<th>UPCR at biopsy (g/gCr)</th>
<th>UPCR at 24-hr urine microproteinuria (g/day)</th>
<th>Hematuria</th>
<th>Last FU</th>
<th>Scr at FU (mg/dL)</th>
<th>UPCR at FU (g/gCr)</th>
<th>Hematuria</th>
</tr>
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<tbody>
<tr>
<td>ATI</td>
<td>71</td>
<td>F</td>
<td>Yes</td>
<td>Hypertension, dyslipidemia</td>
<td>15/40</td>
<td>None</td>
<td>2.65</td>
<td>0.75</td>
<td>1.19</td>
<td>Negative</td>
<td>55</td>
<td>1.19</td>
<td>0.75</td>
<td>Negative</td>
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<tr>
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<td>27</td>
<td>M</td>
<td>Yes</td>
<td>Hypertension, dyslipidemia, liver cirrhosis</td>
<td>23/38</td>
<td>BP control</td>
<td>7.21</td>
<td>0.78</td>
<td>2.98</td>
<td>0.19</td>
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<td>2.89</td>
<td>0.75</td>
<td>No</td>
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<tr>
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<td>26</td>
<td>F</td>
<td>No</td>
<td>None</td>
<td>39/79</td>
<td>Steroid</td>
<td>2.57</td>
<td>2.21</td>
<td>4.13</td>
<td>Yes</td>
<td>No</td>
<td>174</td>
<td>0.58</td>
<td>4.13</td>
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<tr>
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<td>73</td>
<td>M</td>
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<td>Hypertension</td>
<td>27/51</td>
<td>Steroid + CNI</td>
<td>0.51</td>
<td>5.33</td>
<td>5.90</td>
<td>Yes</td>
<td>No</td>
<td>271</td>
<td>0.57</td>
<td>Yes</td>
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<tr>
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<td>F</td>
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<td>None</td>
<td>36/79</td>
<td>Steroid + CNI</td>
<td>0.57</td>
<td>6.90</td>
<td>9.00</td>
<td>Yes</td>
<td>No</td>
<td>532</td>
<td>0.85</td>
<td>Yes</td>
</tr>
<tr>
<td>ATI</td>
<td>61</td>
<td>M</td>
<td>Yes</td>
<td>Hypertension</td>
<td>25/79</td>
<td>Steroid + CNI</td>
<td>0.57</td>
<td>6.90</td>
<td>9.00</td>
<td>Yes</td>
<td>No</td>
<td>532</td>
<td>0.85</td>
<td>Yes</td>
</tr>
<tr>
<td>ATI</td>
<td>53</td>
<td>F</td>
<td>Yes</td>
<td>Hypertension</td>
<td>35/79</td>
<td>Steroid</td>
<td>2.57</td>
<td>2.21</td>
<td>4.13</td>
<td>Yes</td>
<td>No</td>
<td>174</td>
<td>0.58</td>
<td>4.13</td>
</tr>
<tr>
<td>ATI</td>
<td>24</td>
<td>F</td>
<td>No</td>
<td>None</td>
<td>24/79</td>
<td>Steroid + CNI</td>
<td>0.57</td>
<td>6.90</td>
<td>9.00</td>
<td>Yes</td>
<td>No</td>
<td>271</td>
<td>0.57</td>
<td>Yes</td>
</tr>
</tbody>
</table>

**Key:** AKI, acute kidney injury; ARB, angiotensin receptor blocker; ATI, acute tubular injury; BP, blood pressure; CNI, calcineurin inhibitor; COVID-19, coronavirus disease 2019; DOE, dyspnea on exertion; F, female; FSGS, focal segmental glomerulosclerosis; FU, follow-up; HSP, Henoch-Schönlein purpura nephritis; HTN, hypertensive nephropathy; IgAN, immunoglobulin A nephropathy; M, male; MCD, minimal change disease; Scr, serum creatinine; UPCR, urine protein-to-creatinine ratio.
COVID-19 vaccination were recruited from multiple centers. The collected GNs were composed of IgAN (n = 11, 57.9%), HSP (n = 3, 15.8%), FSGS (n = 1, 5.3%), immunoglobulin M nephropathy (n = 1, 5.3%), lupus nephritis (n = 1, 5.3%), pauci-immune crescentic GN (n = 1, 5.3%), and AIN (n = 1, 5.3%). The age at the time of renal biopsy ranged from 18 to 70 years, and the main symptoms were proteinuria, hematuria, edema, and AKI. Both messenger RNA (mRNA)-based vaccines (BNT162b2, n = 8; mRNA-1273, n = 3) and adenovirus-based vaccines (ChAdOx1 nCoV-19, n = 2; Ad26.COV.S, n = 1) were administered. Except for one patient with HSP who developed 32 days after the first dose of ChAdOx1 nCoV-19 and another patient with AIN who developed 7 days after the first dose of BNT162b2, the initial symptoms developed after the second or third dose. The time interval between the first vaccination and symptoms varied from 1 to 50 days. Seven patients experienced symptoms within a short time, even within 1 week after vaccination, while others had a more extended interval. Some patients underwent kidney biopsy more than a year after symptom onset, whereas those who showed acute symptoms underwent kidney biopsy within 1 to 2 months. Most patients received steroids or other immunosuppressive therapies. However, progression to end-stage kidney disease (ESKD) was not observed among the patients during follow-up (Table 2).

**Association with COVID-19 or vaccination in unsuspected kidney biopsies**

From April 2021 to December 2022 at Severance Hospital, 432 patients underwent native kidney biopsies, and 12 patients (2.8%) were included in the multicenter series of GNs following COVID-19 or vaccination. Among the remaining 420 patients whose connection to COVID-19-related events was initially unknown, a survey was successfully conducted to investigate COVID-19 infection or vaccination history in 143 patients. Among 143 patients, 29 (20.3%) had preceding COVID-19 infection. Renal biopsy was conducted on an average of 99.0 days (SD, 62.1 days) after infection, and the most common histological diagnosis was IgAN/HSP. Twenty-seven patients (93.1%) also received vaccinations before tissue examination. The most commonly administered vaccine was BNT162b2. The average interval between vaccination and kidney biopsy was 347 days (Supplementary Table 1, available online).

Eighty-three patients (58.0%, 83 of 143) without a history of COVID-19 received vaccination before biopsy. The BNT162b2 vaccine was the most commonly used vaccine. The median days from the first vaccination to the kidney biopsy was 140.0 (IQR, 77.0–272.0) (Supplementary Table 2, available online). In contrast, 24 individuals (16.8%, 24 of 143) had no history of COVID-19 infection or vaccination before biopsy. There was no significant difference in demographics or pathological diagnoses between the groups with or without a vaccination history, and IgAN/HSP was the most common diagnosis in both groups (Supplementary Table 2, available online).

Because IgAN and HSP were the leading causes of postvaccination GNs in multiple centers, we further focused on postvaccination IgAN/HSP (latent period, <50 days) among the patients with a prior vaccination history by analyzing the duration to symptomatic onset. Nine patients with IgAN/HSP (two patients with a history of IgAN) who developed symptoms within 50 days postvaccination and did not have any history of COVID-19 infection were selected. Seven (77.8%) were vaccinated with BNT162b2, and the other two (22.2%) were vaccinated with Ad26.COV.S. Five patients (55.6%) had their first vaccination before onset, whereas one (11.1%) and two (22.2%) had the second or third doses, respectively. The symptoms typically include hematuria and proteinuria. One patient presented AKI. Two patients received immunosuppressive treatment, and the remaining patients received conservative care for proteinuria. None of the patients developed ESKD (Table 3).

**Biopsy diagnosis of glomerulonephritis cases during the COVID-19 pandemic era in South Korea**

To examine the changes in GN diagnoses during the COVID-19 pandemic, Bayesian structural time-series models and ARIMA models were applied to analyze patients with kidney biopsies from a single institution. There was no significant difference in the frequency of kidney biopsies before and after the initiation of the nationwide vaccine program or the peak of the COVID-19 pandemic (Supplementary Fig. 1, available online). Bayesian structural time-series models showed that compared with the pre-nationwide vaccine program period, the monthly
<table>
<thead>
<tr>
<th>Pathologic diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Underlying diseases</th>
<th>Clinical presentation</th>
<th>Vaccine (before presentation)</th>
<th>Latest dose</th>
<th>Symptom onset time/biopsy time after last vaccination (day)</th>
<th>Scr at biopsy (mg/dL)</th>
<th>UPCR or dipstick at biopsy (g/gCr)</th>
<th>Hematuria</th>
<th>Treatment</th>
<th>Last FU (day)</th>
<th>Scr at FU (mg/dL)</th>
<th>UPCR or dipstick at FU (g/gCr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgAN</td>
<td>24</td>
<td>F</td>
<td>None</td>
<td>Proteinuria, hematuria</td>
<td>BNT162b2</td>
<td>2nd</td>
<td>1/203</td>
<td>0.65</td>
<td>1.36</td>
<td>Yes</td>
<td>Steroid</td>
<td>412</td>
<td>0.78</td>
<td>0.45</td>
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<tr>
<td>IgAN</td>
<td>35</td>
<td>F</td>
<td>None</td>
<td>Proteinuria, hematuria</td>
<td>BNT162b2</td>
<td>2nd</td>
<td>Not known/50 after initial presentation</td>
<td>0.77</td>
<td>0.96</td>
<td>Yes</td>
<td>ARB + ravulizumab</td>
<td>383 after initial presentation</td>
<td>0.80</td>
<td>1.04</td>
</tr>
<tr>
<td>IgAN</td>
<td>47</td>
<td>M</td>
<td>None</td>
<td>Edema</td>
<td>BNT162b2</td>
<td>2nd</td>
<td>32/46</td>
<td>1.13</td>
<td>8.79</td>
<td>No</td>
<td>Steroid → steroid + CNI</td>
<td>532</td>
<td>1.28</td>
<td>2.18</td>
</tr>
<tr>
<td>IgAN</td>
<td>61</td>
<td>F</td>
<td>Ulcerative colitis</td>
<td>Proteinuria, hematuria</td>
<td>BNT162b2</td>
<td>2nd</td>
<td>Not known/63 after initial presentation</td>
<td>1.01</td>
<td>1.37</td>
<td>Yes</td>
<td>ARB</td>
<td>466 after initial presentation</td>
<td>0.88</td>
<td>0.11</td>
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<tr>
<td>IgAN</td>
<td>18</td>
<td>F</td>
<td>None</td>
<td>Hematuria</td>
<td>mRNA-1273</td>
<td>3rd</td>
<td>1/41</td>
<td>0.56</td>
<td>1.33</td>
<td>Yes</td>
<td>ARB + SGLT2i</td>
<td>293</td>
<td>0.57</td>
<td>0.43</td>
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<tr>
<td>IgAN</td>
<td>23</td>
<td>F</td>
<td>None</td>
<td>Hematuria</td>
<td>mRNA-1273</td>
<td>2nd</td>
<td>2/77</td>
<td>0.60</td>
<td>0.76</td>
<td>Yes</td>
<td>Steroid</td>
<td>473</td>
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<td>0.08</td>
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<td>IgAN</td>
<td>65</td>
<td>F</td>
<td>None</td>
<td>Edema, abdominal pain</td>
<td>mRNA-1273</td>
<td>3rd</td>
<td>20/32</td>
<td>0.75</td>
<td>3+</td>
<td>Yes</td>
<td>No treatment</td>
<td>430</td>
<td>0.61</td>
<td>0.04</td>
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<tr>
<td>IgAN</td>
<td>39</td>
<td>F</td>
<td>None</td>
<td>Hematuria</td>
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<td>1st</td>
<td>Not known/55 after initial presentation</td>
<td>0.97</td>
<td>1.61</td>
<td>Yes</td>
<td>ARB + SGLT2i</td>
<td>273 after initial presentation</td>
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<td>54</td>
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<td>Hypertension, dyslipidemia</td>
<td>Proteinuria, hypertension</td>
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<td>2nd</td>
<td>3/283</td>
<td>1.56</td>
<td>2.96</td>
<td>Yes</td>
<td>ARB + SGLT2i</td>
<td>441</td>
<td>1.52</td>
<td>1.61</td>
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<tr>
<td>IgAN</td>
<td>35</td>
<td>F</td>
<td>None</td>
<td>Proteinuria, hematuria</td>
<td>Not known</td>
<td>3rd</td>
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<td>0.70</td>
<td>1.13</td>
<td>Yes</td>
<td>ARB</td>
<td>446</td>
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<td>1.07</td>
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<td>None</td>
<td>Proteinuria, hematuria</td>
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<td>3rd</td>
<td>Not known/not known</td>
<td>0.75</td>
<td>1.01</td>
<td>Yes</td>
<td>ARB</td>
<td>208</td>
<td>0.83</td>
<td>1.63</td>
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<td>41</td>
<td>M</td>
<td>None</td>
<td>Purpura</td>
<td>Ad26.COV.S</td>
<td>2nd</td>
<td>46/286</td>
<td>0.93</td>
<td>0.44</td>
<td>Yes</td>
<td>Steroid + azathioprine</td>
<td>407</td>
<td>0.97</td>
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<td>HSP</td>
<td>59</td>
<td>F</td>
<td>None</td>
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<td>1st</td>
<td>32/42</td>
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<td>2.65</td>
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<td>512</td>
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<td>19</td>
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<td>Yes</td>
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<td>103</td>
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<td>48</td>
<td>M</td>
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<td>Nephrotic syndrome</td>
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<td>None</td>
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<td>1.81</td>
<td>4.82</td>
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<td>Pauci-immune crescentic GN</td>
<td>70</td>
<td>F</td>
<td>None</td>
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<td>mRNA-1273</td>
<td>3rd</td>
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<td>-</td>
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</table>

AIN, acute interstitial nephritis; ARB, angiotensin receptor blocker; AKI, acute kidney injury; CNI, calcineurin inhibitor; COVID-19, coronavirus disease 2019; CYC, cyclophosphamide; F, female; FSGS, focal segmental glomerulosclerosis; FU, follow-up; GN, glomerulonephritis; HSP, Henoch-Schönlein purpura; IgAN, immunoglobulin A nephropathy; IgMN, immunoglobulin M nephropathy; LN, lupus nephritis; M, male; MMF, mycophenolate mofetil; Scr, serum creatinine; SGLT2i, sodium-glucose cotransporter-2 inhibitor; UPCR, urine protein-to-creatinine ratio.
<table>
<thead>
<tr>
<th>Pathologic diagnosis</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>M</th>
<th>E</th>
<th>S</th>
<th>T</th>
<th>C</th>
<th>Clinical presentation</th>
<th>Vaccine regimen</th>
<th>Dose</th>
<th>Symptom onset time/ biopsy time after last vaccination (day)</th>
<th>Scr at biopsy (mg/dL)</th>
<th>UPCR at biopsy (g/gCr)</th>
<th>Hematuria</th>
<th>Treatment</th>
<th>Last FU after biopsy (day)</th>
<th>Scr at FU (mg/dL)</th>
<th>UPCR at FU (g/gCr)</th>
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<td>0</td>
<td>0</td>
<td>Hematuria</td>
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<td>1st</td>
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<td>Aggravated proteinuria, known IgAN)</td>
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<td>Hematuria, proteinuria</td>
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<td>34/84</td>
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<td>Yes</td>
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M, mesangial hypercellularity; E, endocapillary hypercellularity; S, segmental sclerosis; T, interstitial fibrosis/tubular atrophy; C, crescent; AKI, acute kidney injury; ARB, angiotensin receptor blocker; F, female; FU, follow-up; HSP, Henoch-Schönlein purpura; IgAN, immunoglobulin A nephropathy; M, male; MMF, mycophenolate mofetil; Scr, serum creatinine; SGLT2i, sodium-glucose cotransporter-2 inhibitor; UPCR, urine protein-to-creatinine ratio.

Oxford classification: M, mesangial hypercellularity; E, endocapillary hypercellularity; S, segmental sclerosis; T, interstitial fibrosis/tubular atrophy; C, crescent.
proportion of AIN, nonspecific histology, podocytopathy, and pauci-immune GN were changed as follows: the proportion of AIN, nonspecific histology and podocytopathy increased by 0.96 (95% confidence interval [CI], −0.11 to 2.00), 2.51 (95% CI, 0.37–4.60), and 5.12 (95% CI, 0.80–9.30), respectively. In contrast, the proportion of pauci-immune GN decreased by −3.71 (95% CI, −0.64 to −1.00) (Fig. 2). Based on the analysis, we found a posterior probability of a causal effect ≥ 95%, indicating a strong causal relationship in the AIN, nonspecific histology, podocytopathy, and pauci-immune GN categories (Supplementary Table 3, available online). Other diseases showed no differences in diagnosis rates before and after the nationwide vaccination program (posterior probability, <95%) (Supplementary Table 3, available online; Fig. 2). Supplementary Fig. 2 (available online) and Supplementary Table 4 (available online) present the predictions of the ARIMA model. Other nephropathy and podocytopathy entities showed significant step changes in the diagnostic rates: −1.627 (95% CI, −2.904 to −0.349) and 0.827 (95% CI, 0.168–1.485), respectively. AIN showed a significant slope change in the diagnosis rate (0.049 [95% CI, 0.013–0.084]). However, no disease was significant for either step or slope changes.

Compared with the pre-pandemic period, only the monthly proportion of AIN was significantly changed in a Bayesian structural time-series model (absolute effect, 3.09 [95% CI, 1.70 to 4.50]; posterior probability of a causal effect, ≥95%) (Supplementary Table 5 and Supplementary Fig. 3; available online). In the ARIMA model, no disease showed a significant difference in either step change or slope change in diagnosis rates (Supplementary Table 6, Supplementary Fig. 4; available online).

**Discussion**

In this study, which included patients from multiple centers in South Korea, we showed for the first time the spectrum of new-onset kidney disease occurring shortly after COVID-19 infection or vaccination in South Korea. We also determined whether the incidence of GN types changed during the COVID-19 pandemic. Understanding the prevalence and characteristics of COVID-19-related renal disease is critical for monitoring the safety and efficacy of vaccination. More importantly, the results of our study provide insights into the impact of COVID-19-related events on the management of patients with renal disease.

Renal dysfunction is a frequent complication that affects 5% to 37% of hospitalized COVID-19 patients [3]. COVID-19-induced AKI and glomerular disease pathogenesis involve various mechanisms, including hypoperfusion, cytokine storm, endothelial injury, and potential direct kidney infection [3]. In our case series, COVID-19-infected patients displayed diverse glomerular diseases and frequently presented with AKI, likely attributable to COVID-19 [14]. The most frequently observed glomerular disorder was podocytopathy, comprising one case of collapsing variant FSGS and two cases of MCD. The FSGS case exhibited diffuse and severe effacement of the podocyte foot processes, indicating primary FSGS. Collapsing variant FSGS (collapsing glomerulopathy) is the most common type of GN observed in COVID-19 patients. It is believed to be induced by immune dysregulation triggered by COVID-19 [14]. In addition, we identified newly diagnosed IgAN and HSP in COVID-19 patients. It is plausible to assume that COVID-19 upregulates IgA or unmask subclinical IgAN during the course of the disease, potentially contributing to the development of IgAN and HSP [14,20].

As large-scale vaccination against COVID-19 is underway, concerns about kidney disease that occurs after vaccination have been raised. More than 160 cases of de novo or relapsing GNs after COVID-19 vaccines have been reported [9,21,22]. In our series, the majority of postvaccination GNs (94.7%) manifested as glomerular disease, except for one case of AIN. IgAN (57.9%) and HSP (15.8%) were the most common types of GNs. Among the previously reported GNs that occurred after COVID-19 vaccination [22,23], IgAN was the most common, accounting for 32.6% of the total cases. This trend was also identified in our series where IgAN (57.9%) and HSP (15.8%) were frequent among postvaccination GNs. However, a recent study from Germany reported only one case of IgAN (3.7%) among GNs following a COVID-19-related event [21]. Ethnic differences may be ascribed to the inconsistencies in IgAN occurrence in vaccinated patients [24]. The present study also concurred with previous studies in that postvaccination GNs did not have distinctive features in terms of clinicopathological characteristics [9,25], although the number of patients was too limited to draw a conclusion. The vaccine platforms used against COVID-19 activate diverse immune responses; in particular, mRNA vaccines are known to vigorously
Figure 2. The monthly trend of glomerulonephritis after the initiation of nationwide vaccine program (after April 2021) in Severance Hospital based on the Bayesian structural time-series models. (A) Total, (B) acute tubular injury (AIN), (C) acute interstitial nephritis (ATI), (D) immunoglobulin A nephropathy (IgAN), (E) membranous nephropathy (MN), (F) nonspecific histology, (G) other nephropathies, (H) immune complex-mediated glomerulonephritis (IC-GN), (I) pauci-immune crescentic glomerulonephritis, (J) podocytopathy, and (K) thrombotic microangiopathy (TMA). The solid lines denote the observed value, the blue-dotted lines denote the predicted value, and the blue shades denote 95% confidence intervals of the predicted value.
enhance immune reactions, which makes it reasonable to consider that COVID-19 vaccines might trigger GNs [4]. While, other reports have challenged this hypothesis. In Switzerland, the incidence of GNs during local vaccination programs was similar to that during the baseline period [25], and a cohort of patients with IgAN did not experience gross hematuria after receiving mRNA-based COVID-19 vaccines [26]. Therefore, the exact risk of developing GNs following COVID-19 vaccination is unclear and is probably very low, emphasizing the benefits of vaccination [10].

In this study, we retrospectively examined the COVID-19 vaccination and infection history of patients who underwent renal biopsy to investigate their relevance to GN. Although it was not possible to survey all patients who underwent renal biopsy, there was no clear evidence of an association between COVID-19 or vaccination history and specific glomerular diseases. Most patients with a history of COVID-19 or vaccination were diagnosed with IgAN. The frequency of IgAN did not appear to change after the pandemic, even when examining the correlation between COVID-19–related history (vaccination or infection) and renal biopsy. However, the worsening of proteinuria after vaccination in patients with preexisting IgAN in this study is consistent with the results of previous studies, suggesting that COVID-19 vaccination may affect IgAN [20,27,28].

The proportion of glomerular diseases increased or decreased significantly after the COVID-19 vaccination program or pandemic. Our Bayesian structural time-series models showed a significant increase in the diagnostic rate of AIN after initiating a nationwide vaccination program or pandemic, supporting the findings of previous case reports [29–31]. AIN after COVID-19 is believed to be a direct effect of a virus-induced immune mechanism or an indirect effect of other conditions associated with COVID-19 [31]. A Bayesian structural time-series model also reported a decrease in the proportion of pauci-immune crescentic GN after the nationwide vaccination program. In contrast, among all kidney biopsies in Germany, no definite increase or decrease in the frequency of pauci-immune crescentic GN was observed during the prepandemic, pandemic, and vaccination periods [21]. Because cases of pauci-immune crescentic GN have been observed after COVID-19 infection or vaccination [32,33], changes in the frequency of pauci-immune crescentic GN should be interpreted with caution. In addition, our results showed that the proportion of patients with nonspecific histology and podocytopathy increased after the nationwide vaccination program. However, because none of the GN showed a significant change in the diagnosis rate in either Bayesian structural time-series models or ARIMA models, it would be difficult to conclude whether the COVID-19 pandemic or nationwide vaccination program had an impact on the incidence of glomerular disease.

This study had some limitations. First, the changes in the diagnosis rate of GN and the investigation of COVID-19–related history among patients undergoing renal biopsy were conducted only at a single center. However, this single-center investigation may have strengths, particularly in terms of ensuring consistency of kidney biopsy and pathologic diagnostic criteria. Second, the pandemic may have affected patients’ utilization of healthcare facilities, leading to potential inaccuracies in the diagnosis rate. However, in South Korea, no strict lockdown fundamentally restricted patients’ access to healthcare facilities. Lastly, COVID-19–related histories obtained through surveys at outpatient clinics can be inaccurate and occasionally rely on patient memory. Nevertheless, there are no available retrospective data from Korea investigating the history of COVID-19 infection or vaccination among patients who have already undergone native renal biopsy.

In summary, this is the first comprehensive report in South Korea characterizing biopsy-proven kidney diseases following COVID-19 or vaccination. This study revealed a spectrum of glomerular and tubulointerstitial diseases, with podocytopathy and IgAN being the leading types postinfection and postvaccination, respectively. The incidence of GNs has changed during the pandemic; however, this requires further investigation. Monitoring and understanding COVID-19–related renal diseases are crucial for effective patient management and vaccination efforts.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

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data collection, or analysis.

**Data sharing statement**

The data presented in this study are available upon reasonable request from the corresponding author.

**Authors' contributions**

Conceptualization: MJ
Data curation, Formal analysis: All authors
Writing–original draft: HWK, MJ
Writing–review & editing: HWK, MJ
All authors read and approved the final manuscript.

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Minsun Jung, https://orcid.org/0000-0002-8701-4282

**References**


Vitamin D and narrowband ultraviolet B phototherapy for chronic kidney disease-associated pruritus

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Background: In addition to improving the serum vitamin D balance, narrowband ultraviolet B (NB-UVB) phototherapy can effectively treat chronic kidney disease-associated pruritus (CKD-aP). We investigated the degree of CKD-aP amelioration according to changes in the serum vitamin D level after NB-UVB phototherapy.

Methods: This was a before–after clinical study in patients with refractory CKD-aP on hemodialysis. NB-UVB phototherapy was administered thrice weekly for 12 weeks. The response of CKD-aP to NB-UVB phototherapy was assessed as the change in pruritus intensity over time. A rapid response was defined as a reduction in the visual analog scale (VAS) score of ≥50% within the first 6 weeks of NB-UVB phototherapy.

Results: We included 34 patients in this study. Although serum 25-hydroxy vitamin D [25(OH)D] concentrations increased significantly, by a median of 17.4 ng/mL, after the phototherapy course, other serologic parameters did not change. NB-UVB phototherapy reduced the VAS score for pruritus intensity over time significantly more in patients with Δ25(OH)D of >17.4 ng/mL than in patients with Δ25(OH)D of ≤17.4 ng/mL (p = 0.001). Ten patients were rapid responders. Multivariate logistic regression analysis showed that Δ25(OH)D was independently associated with rapid response (odds ratio, 1.29; 95% confidence interval, 1.02–1.63; p = 0.04).

Conclusion: The effect of NB-UVB phototherapy on patients with CKD-aP correlated with their increase in serum vitamin D levels. Further well-designed clinical and experimental studies are needed to clarify the relationship between NB-UVB phototherapy and serum vitamin D levels in patients with CKD-aP.

Keywords: Chronic renal insufficiency, Phototherapy, Pruritus, Vitamin D

Introduction

Chronic kidney disease-associated pruritus (CKD-aP), also known as uremic pruritus, is defined as itching that is directly related to kidney disease without other comorbidities that could cause the itching. It usually presents as itching across large bilaterally symmetrical surface areas without a dermatomal pattern, with daily or near-daily occurrence [1]. In addition, it can manifest as localized itching on the back, face, or arms [2,3]. CKD-aP causes adverse effects on sleep quality, mood, and social functions and is independently associated with mortality [2,4]. Although its pathophysiology is incompletely understood, the known predisposing factors for its development include increased blood urea nitrogen, hyperphosphatemia, and hyperparathyroidism [5–7]. Other contributing factors include ane-
mia, elevated ferritin, low transferrin, and low albumin levels [8]. Therefore, although the mechanisms of CKD-aP are poorly understood, metabolic disequilibrium as a result of renal failure could be a cause.

Despite advances in hemodialysis patient care during the past few decades, CKD-aP remains a major clinical symptom and medical challenge. Resistant pruritus is defined as an itch lasting >4 weeks despite adequate dialysis, optimization of metabolic parameters, and the use of topical emollients and analgesics [9]. Such patients can be treated with oral antihistamines; if pruritus persists after 1 to 2 weeks of that treatment, they can be treated with gabapentin or pregabalin. Most patients with CKD-aP partially respond to emollients, topical analgesics, oral antihistamines, or gabapentin [9]. For patients who are refractory to these agents, narrowband ultraviolet B (NB-UVB) phototherapy is an effective therapeutic choice [9]. The benefits of NB-UVB phototherapy are thought to occur through modulation of the inflammatory state of these patients [10]. However, only small or uncontrolled clinical studies have been conducted on the effect of NB-UVB phototherapy on CKD-aP.

A low serum vitamin D level is associated with the metabolic disequilibrium commonly observed in patients undergoing hemodialysis [11,12], possibly because of reduced skin synthesis of vitamin D [13]. These patients are likely exposed to less sunlight than average [14,15], and uremia can blunt the response of serum vitamin D to ultraviolet B (UVB) irradiation [13]. Moreover, hyperpigmentation, a common cutaneous manifestation in these patients, could play an additional role in impairing the endogenous synthesis of vitamin D [14,16]. Therefore, NB-UVB phototherapy could be a way to improve the serum vitamin D balance in patients undergoing hemodialysis. Ala-Houhala et al. [17] showed that NB-UVB phototherapy significantly improved serum vitamin D balance in these patients. Meanwhile, inflammatory skin disease is related to low serum vitamin D levels [18,19]. Additionally, vitamin D supplementation reduces disease activity in patients with inflammatory skin disease [20–23]. Taken together and considering CKD-aP as a skin counterpart of chronic inflammation in patients undergoing hemodialysis [24], the benefit of NB-UVB phototherapy on CKD-aP might correlate with improved serum vitamin D levels.

As an effective treatment choice for refractory CKD-aP, NB-UVB phototherapy has been postulated to improve serum vitamin D levels, in addition to modulating the inflammatory state. However, data on serum vitamin D levels and NB-UVB phototherapy in CKD-aP are lacking. Therefore, we investigated the degree of CKD-aP amelioration and changes in serum vitamin D levels after NB-UVB phototherapy in patients undergoing hemodialysis.

Methods

Ethics statement

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board of Kangdong Sacred Heart Hospital (No. 2018-01-008). Written informed consent was obtained from all patients prior to enrollment. This study was registered at Clinical Research Information Service (http://cris.nih.go.kr; KCT0003701).

Patients

This study was conducted at Kangdong Sacred Heart Hospital between March 2018 and December 2020. The inclusion criteria were as follows: 1) age of >18 years; 2) undergoing hemodialysis thrice a week for >3 months; 3) amount of dialysis delivered (Kt/V urea; K = clearance of urea, t = time on dialysis, V = estimated total body water) of >1.2; 4) hemoglobin (Hb) level of >10 g/dL; 5) pruritus with an intensity of ≥5 measured on a visual analog scale (VAS); 6) pruritis for ≥2 months; and 7) no response to oral antihistamines, gabapentin or pregabalin, or topical emollients. Of note, if the pruritus did not improve after 4 weeks of topical emollients, patients took oral antihistamines for 2 weeks. After that, the patients whose pruritus did not improve took gabapentin or pregabalin for 2 weeks. If the pruritus still did not improve, it was defined as no response. The exclusion criteria were a history of photosensitivity, active infection, psychotic illness or other communication disability, primary skin disorder, cholestatic liver disease or acute hepatitis, and active malignancy.

Phototherapy and protocol

NB-UVB phototherapy was administered to the whole body
surface in an ultraviolet irradiation cubicle (HOUVA-II; National Biological Corp.) incorporating Phillips TL-01 lamps with a peak wavelength emission of 311 nm. Phototherapy was conducted thrice weekly for 12 weeks, for a total of 36 sessions. The initial dose of NB-UVB was 280 mJ/cm², which is approximately 70% of the minimal erythema dose. The phototherapy dose was generally increased by 10% to 15% per session, as tolerated. According to the response, the phototherapy dose was adjusted as follows: maintaining the previous dose if asymptomatic erythema appeared for 24 hours; reducing the dose by 10% if erythema was accompanied by mild pain or pruritus; restarting from one-third of the dose after recovery if painful erythema or bullae formation associated with mild pain or pruritus occurred.

Study design, data collection, and outcome measures

This was a before-and-after study design. At baseline, we evaluated the intensity of the pruritus using the VAS score (0 [no pruritus] to 10 [most severe pruritus]). The VAS score was evaluated each time the patients received NB-UVB phototherapy. Laboratory parameters of serum Hb, ferritin, transferrin saturation (TSAT), intact parathyroid hormone (iPTH), 25-hydroxy vitamin D [25(OH)D], calcium, phosphate, C-reactive protein (CRP), and Kt/V values were measured at baseline and after 12 weeks. If the patient did not complete the phototherapy protocol, laboratory parameters were measured at the next dialysis session after the end of phototherapy.

Vitamin D deficiency and insufficiency were defined as serum 25(OH)D concentrations of <20 ng/mL and 20–30 ng/mL, respectively. The Δ25(OH)D was calculated as the difference between the serum 25(OH)D concentration at 12 weeks or the last follow-up day and that at baseline. The response of CKD-aP to NB-UVB phototherapy was assessed by the change in the intensity of pruritus over time, with rapid response defined as a reduction in the VAS score of ≥50% within the first 6 weeks.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 27.0 (IBM Corp.) and R version 3.6.1 (R Foundation for Statistical Computing). Differences before and after NB-UVB phototherapy were analyzed using the Wilcoxon signed-rank test. Continuous variables are expressed as means ± standard deviations, and categorical variables are expressed as numbers (percentages). Differences between groups were assessed using Student t test, the chi-square test, or Fisher exact test.

Changes in VAS scores over time were compared between groups using a linear mixed model analysis. A logistic regression analysis was performed to assess variables associated with a rapid response. A multivariate analysis was performed using all covariates with p-values of <0.1 in the univariate analyses. Even if their p-values were >0.1, potential confounding factors were included in the multivariate analysis. All probabilities were two-tailed, and statistical significance was set at p < 0.05.

Results

Study patients

Initially, 35 patients enrolled in the study. One patient dropped out immediately (after the first session) because she could not tolerate the heat in the cabinet during phototherapy. Therefore, 34 patients with at least one follow-up visit were included in the statistical analyses. Two and three patients withdrew from the phototherapy protocol within 6 weeks and after 6 weeks, respectively. Thus, 29 patients completed the phototherapy protocol. None of the withdrawals were due to a lack of efficacy. A flowchart of the patients is shown in Fig. 1.

Narrowband ultraviolet B phototherapy and change in serum vitamin D

Prior to NB-UVB phototherapy, the median serum 25(OH)D concentration was 10.9 ng/mL (interquartile range [IQR], 5.3–26.0 ng/mL) in all patients. Among them, 23 and 8 patients had vitamin D deficiency and insufficiency, respectively. After the last NB-UVB phototherapy session, the median serum 25(OH)D concentration was 32.5 ng/mL (IQR, 25.8–39.3 ng/mL). That increase was statistically significant (p < 0.001), and the serum 25(OH)D concentration increased by a median of 17.4 ng/mL (IQR, 11.0–22.4 ng/mL). At that time, four and seven patients had vitamin D deficiency and insufficiency, respectively.

Notably, serum iPTH and calcium concentrations did
not change after the NB-UVB phototherapy course. In addition, the serum Hb, ferritin, TSAT, phosphate, CRP, and Kt/V values were unchanged (Table 1).

Baseline characteristics according to the change in serum vitamin D

The subjects’ median age was 62 years (IQR, 56–69 years), and 14 patients (41.2%) were male. The median dialysis duration was 52.5 months (IQR, 29.6–107.4 months). The underlying cause of end-stage renal disease (ESRD) was diabetes in 23 patients (67.6%). Notably, none of the patients required nutritional vitamin D. When the patients were divided into two groups according to the median Δ25(OH)D level, serum iPTH concentrations were significantly higher in patients with Δ25(OH)D of ≤17.4 ng/mL than in those with Δ25(OH)D of >17.4 ng/mL. However, the groups did not differ with respect to age, sex, dialysis duration, underlying cause of ESRD, VAS score, cumulative dose of NB-UVB phototherapy, serum Hb concentration, ferritin concentration, TSAT, 25(OH)D concentration, calcium concentration, phosphorus concentration, CRP level, or Kt/V. To control secondary hyperparathyroidism, 13 patients used synthetic vitamin D analogues (calcitriol in eight and paricalcitol in five). The proportion of patients receiving synthetic vitamin D analogues did not differ between patients with Δ25(OH)D of ≤17.4 ng/mL and those with Δ25(OH)D of >17.4 ng/mL (Table 2). Of note, not all patients used vitamin D supplementation.

Response to narrowband ultraviolet B phototherapy according to the change in serum vitamin D

NB-UVB phototherapy reduced the VAS score for pruritus intensity more significantly in patients with Δ25(OH)D of >17.4 ng/mL than in patients with Δ25(OH)D of ≤17.4 ng/mL (p = 0.001) (Fig. 2). Notably, NB-UVB phototherapy significantly improved the VAS score for pruritus intensity over time in all patients (p < 0.001) (Fig. 2). Among them, 10 exhibited a rapid response. The logistic regression analysis

Table 1. Serologic parameters before the first NB-UVB phototherapy session and after the last NB-UVB phototherapy session in 34 patients with refractory CKD-aP

<table>
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<th>Parameter</th>
<th>Before the first NB-UVB phototherapy</th>
<th>After the last NB-UVB phototherapy</th>
<th>p-value</th>
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<td>10.1 ± 0.3</td>
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</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>126.5 (88.6–201.5)</td>
<td>129.5 (97.8–190.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>26.7 (19.0–33.3)</td>
<td>25.8 (18.9–34.9)</td>
<td>0.30</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>234.0 (162.0–468.8)</td>
<td>319.0 (280.0–442.5)</td>
<td>0.26</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>10.9 (5.3–26.0)</td>
<td>32.5 (25.8–39.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.7 ± 0.5</td>
<td>8.6 ± 0.5</td>
<td>0.16</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.7 ± 1.5</td>
<td>5.6 ± 1.1</td>
<td>0.15</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.5 (0.5–1.3)</td>
<td>0.5 (0.5–1.2)</td>
<td>0.17</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or median (interquartile range).

CKD-aP, chronic kidney disease-associated pruritus; CRP, C-reactive protein; NB-UVB, narrowband ultraviolet B; PTH, parathyroid hormone; TSAT, transferrin saturation; 25(OH)D, 25-hydroxy vitamin D.

*K = clearance of urea, t = time on dialysis, V = estimated total body water.
showed that Δ25(OH)D was independently associated with a rapid response (odds ratio [OR], 1.20; 95% confidence interval [CI], 1.05–1.38; p = 0.01). The effect of Δ25(OH)D on rapid response remained significant even after adjustment for levels of serum Hb, ferritin, TSAT, iPTH, 25(OH)D, calcium, phosphate, CRP, and Kt/V (OR, 1.29; 95% CI, 1.02–1.63; p = 0.04) (Table 3).

**Discussion**

This pre–post clinical study investigated the degree of CKD-aP amelioration according to changes in serum vitamin D levels after NB-UVB phototherapy in patients undergoing hemodialysis. Similar to previous studies, we found that NB-UVB phototherapy was an effective treatment for CKD-aP. We also found that the change in serum 25(OH)D concentration after NB-UVB phototherapy correlated significantly with rapid improvement of refractory CKD-aP.

CKD-aP is considered to be a systemic rather than a local skin disorder [24,25]. Various serological parameters have been identified as potential pruritogens in chronic kidney disease. Parathyroid hormone (PTH) is a possible pathogenic serologic parameter based on the observation that persistent pruritus improves after parathyroidectomy [6,7]. In addition, hypercalcemia and hyperphosphatemia with secondary calcium phosphate crystal deposits in the skin are possible mechanisms [26]. However, PTH did not elicit any cutaneous reaction upon intradermal application in humans, and it was not detected in skin biopsies of affected patients.
Table 3. Factors associated with a rapid response to NB-UVB phototherapy in CKD-aP

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>1.05 (0.97–1.13)</td>
<td>0.23</td>
</tr>
<tr>
<td>Male sex</td>
<td>4.00 (0.70–22.90)</td>
<td>0.12</td>
</tr>
<tr>
<td>HD duration (mo)</td>
<td>0.99 (0.97–1.01)</td>
<td>0.37</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.40 (0.42–13.90)</td>
<td>0.33</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.99 (0.07–14.35)</td>
<td>0.99</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>1.00 (0.99–1.01)</td>
<td>0.95</td>
</tr>
<tr>
<td>TSAT (%)</td>
<td>1.01 (0.97–1.05)</td>
<td>0.69</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>0.99 (0.99–1.00)</td>
<td>0.17</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>1.01 (0.94–1.08)</td>
<td>0.93</td>
</tr>
<tr>
<td>Δ25(OH)D* (ng/mL)</td>
<td>1.20 (1.05–1.38)</td>
<td>0.01</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>1.41 (0.34–5.86)</td>
<td>0.64</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>0.52 (0.49–1.43)</td>
<td>0.52</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.90 (0.63–1.29)</td>
<td>0.56</td>
</tr>
<tr>
<td>Kt/V</td>
<td>2.41 (0.08–74.84)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

CI, confidence interval; CKD-aP, chronic kidney disease-associated pruritus; CRP, C-reactive protein; NB-UVB, narrowband ultraviolet B; HD, hemodialysis; PTH, parathyroid hormone; TSAT, transferrin saturation; 25(OH)D, 25-hydroxy vitamin D.

*Δ25(OH)D, difference between the serum 25(OH)D concentration at 12 weeks or the last follow-up day and that at baseline.

Figure 2. Comparison of VAS scores of pruritus intensity over time during a course of narrowband ultraviolet B phototherapy between patients with Δ25(OH)D of >17.4 ng/mL and patients with Δ25(OH)D of ≤17.4 ng/mL.

VAS, visual analog scale; 25(OH)D, 25-hydroxy vitamin D; Δ25(OH)D, difference between the serum 25(OH)D concentration at 12 weeks or the last follow-up day and that at baseline.

patients [27]. In addition, little clinical evidence supports an association between CKD-aP and higher calcium and phosphate levels [17]. Interestingly, Gilchrest et al. [28] showed that the response of CKD-aP to UVB phototherapy was unaffected by secondary hyperparathyroidism. In line with those findings, we found that a rapid response to NB-UVB phototherapy in refractory CKD-aP did not correlate with iPTH, calcium, or phosphate levels.

Systemic inflammation might play a role in CKD-aP [24,25]. Serological inflammatory parameters, including increased white blood cell count, low albumin level, and high ferritin level, are reportedly associated with CKD-aP [8,29]. In addition, an experimental study showed that UVB radiation could modulate the immune system [10]. Interestingly, Gilchrest et al. [30] showed that tanning patients with UVB phototherapy led to CKD-aP relief, and the effect was maintained even when only half of the body was irradiated. Taken together, these findings suggest that UVB radiation has a systemic effect in modulating the inflammatory state of CKD-aP. In this study, NB-UVB phototherapy significantly reduced CKD-aP intensity. However, the serum ferritin and CRP concentrations did not change after the course of NB-UVB phototherapy. In addition, rapid response to NB-UVB phototherapy in refractory CKD-aP did not correlate.
with ferritin or CRP levels.

Special attention has been focused on imbalances in the expression of mu (μ)- and kappa (κ)-opioid receptors, which cause pruritus [31,32]. Thus, pruritus is increased by μ-receptor activation and κ-receptor blockade and decreased by κ-receptor activation and μ-receptor blockade [33]. This opioid hypothesis is supported by the observation that the ratio of the μ-receptor agonist (beta-endorphin) to the κ-receptor agonist (dynorphin-A) is higher in hemodialysis patients than in healthy controls, and this ratio increases with the severity of pruritus [34]. Meanwhile, UVB light is reportedly responsible for the formation of vitamin D-generated beta-endorphin [35–37]. Therefore, the opioid hypothesis cannot explain the effect of NB-UVB therapy on CKD-aP.

Recent research on CKD-aP points toward the dysregulation of cutaneous innate immunity. Vitamin D plays an important role in the cutaneous innate immune response by regulating the production of the antimicrobial peptide cathelicidin, which is associated with the complex pathogenesis of several chronic inflammatory skin diseases [38]. In addition, vitamin D is considered to be a calcium-regulating hormone in the skin because it regulates the calcium gradient, which is essential for keratinocyte differentiation, in the upper layers of the skin [39]. Momose et al. [40] demonstrated that disruption of the calcium gradient in the upper layers of the skin was associated with CKD-aP. Therefore, vitamin D might adjust the abnormal calcium gradient and consequently regulate abnormal keratinocyte differentiation and reduce the xerosis commonly observed in the skin of CKD-aP patients. Although the precise mechanisms to explain the association between vitamin D and CKD-aP have not been established, the function of vitamin D on keratinocytes might also be related to the underlying pathogenesis. Several clinical studies have shown that patients with inflammatory skin disease have significantly lower serum vitamin D levels than controls [18,19]. In addition, a high dose of vitamin D supplementation reduces disease activity in patients with inflammatory skin disease [20–23].

Vitamin D can be generated in the skin during exposure to sunlight or obtained from diet and supplements. Sun exposure is the primary source of vitamin D in humans. Specifically, the UVB portion of natural sunlight (spectral range, 290–320 nm) generates previtamin D from 7-dehydrocholesterol (provitamin D) in the upper layers of the skin. That previtamin D undergoes a slow temperature-dependent thermal isomerization into vitamin D. Vitamin D is then metabolized into 25(OH)D in the liver [41]. Indeed, several clinical studies have shown that both artificial UVB sources, broadband-UVB and NB-UVB, can lead to 25(OH)D synthesis [17,41–43]. Interestingly, in this study, rapid improvement of refractory CKD-aP after UVB phototherapy correlated significantly with Δ25(OH)D.

Our study has several limitations. First, similar to other clinical studies on CKD-aP, this study was small and uncontrolled. However, we are the first to evaluate the degree of CKD-aP amelioration based on changes in serum vitamin D levels after NB-UVB phototherapy in patients undergoing hemodialysis. Second, this study would have been much more persuasive if we had used other scales, such as the Pruritus Numerical Rating, Peak Pruritus Numerical Rating, Worst Itch Numeric Rating, or 0–21-point itch severity, instead of the VAS to measure pruritus intensity. Third, we did not perform a skin biopsy to evaluate changes in microinflammation and vitamin D in the skin of patients with CKD-aP before and after the course of NB-UVB phototherapy. Fourth, based on the opioid hypothesis, measures of beta-endorphin and dynorphin-A are required to explain the effect of NB-UVB phototherapy on CKD-aP; this study did not measure those parameters.

Despite those limitations, this study has shown that the effect of NB-UVB phototherapy on CKD-aP might correlate with improved serum vitamin D levels. Further well-designed clinical and experimental studies are needed to clarify the relationship between NB-UVB phototherapy and serum vitamin D levels in CKD-aP patients.

**Conflicts of interest**

All authors have no conflicts of interest to declare.

**Funding**

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Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors’ contributions

Conceptualization, Project administration: JO, DHS
Data curation: YKK, HJJ, JO
Formal analysis: YKK, HJJ
Investigation: HJJ
Methodology, Visualization: YKK
Supervision: DHS
Writing–original draft: YKK, DHS
Writing–review & editing: JO, DHS
All authors read and approved the final manuscript.

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References


Role of APE1/Ref-1 in hydrogen peroxide-induced apoptosis in human renal HK-2 cells

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Background: Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multipotent protein that plays essential roles in cellular responses to oxidative stress.

Methods: To examine the role of APE1/Ref-1 in ischemia-reperfusion (I/R) injuries and hydrogen peroxide (H₂O₂)-induced renal tubular apoptosis, we studied male C57BL6 mice and human proximal tubular epithelial (HK-2) cells treated with H₂O₂ at different concentrations. The colocalization of APE1/Ref-1 in the proximal tubule, distal tubule, thick ascending limb, and collecting duct was observed with confocal microscopy. The overexpression of APE1/Ref-1 with knockdown cell lines using an APE1/Ref-1–specific DNA or small interfering RNA (siRNA) was used for the apoptosis assay. The promoter activity of nuclear factor kappa B (NF-κB) was assessed and electrophoretic mobility shift assay was conducted.

Results: APE1/Ref-1 was predominantly localized to the renal tubule nucleus. In renal I/R injuries, the levels of APE1/Ref-1 protein were increased compared with those in kidneys subjected to sham operations. The overexpression of APE1/Ref-1 in HK-2 cells enhanced the Bax/Bcl-2 ratio as a marker of apoptosis. Conversely, the suppression of APE1/Ref-1 expression by siRNA in 1-mM H₂O₂-treated HK-2 cells decreased the Bax/Bcl-2 ratio, the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, p38, c-Jun N-terminal kinase (JNK) 1/2, and NF-κB. In HK-2 cells, the promoter activity of NF-κB increased following H₂O₂ exposure, and this effect was further enhanced by APE1/Ref-1 transfection.

Conclusion: The inhibition of APE1/Ref-1 with siRNA attenuated H₂O₂-induced apoptosis through the modulation of mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 and the nuclear activation of NF-κB and proapoptotic factors.

Keywords: APE1/Ref-1, APE1/Ref-1 inhibitor, Apoptosis, HK-2 cells, Reperfusion injury

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Introduction

Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a ubiquitously expressed multipotent protein that plays an essential role in cellular response to oxidative stress. APE1/Ref-1 (also known as HAP1 or APEX) is a protein composed of 318 amino acids including a redox region and a DNA repair region. It is an essential component in maintaining genome stability, serving as an essential master regulator of the stress response [1]. APE1/Ref-1 is a dual-function protein involved both in the base excision repair pathways of DNA lesions, acting as the major apurinic/apyrimidinic endonuclease, as well as in the eukaryotic transcriptional regulation of gene expression [2,3]. APE1/Ref-1 functions to activate many transcription factors including p53, nuclear factor kappa B (NF-κB), hypoxia-inducible factor (HIF) 1-α, and paired box 5 [2,4]. Moreover, APE1/Ref-1 may be important in the cellular response to oxidative changes triggered during apoptosis. APE1/Ref-1 is localized predominantly in the nucleus and has also been detected in the mitochondria and cytoplasm [5]. The movement of APE1/Ref-1 from the nucleus to the cytoplasm is mediated by an N-terminal nuclear export signal and a C-terminal nuclear localization signal (NLS) [6,7]. Importantly, this subcellular localization is dynamically regulated. APE1/Ref-1 rapidly translocates from the nucleus to the cytoplasm in response to reactive oxygen species (ROS) [8–10]. These two biological activities are located in two functionally distinct domains. The N-terminus, containing the NLS region, is principally devoted to redox activity through Cys65, while the C-terminus exerts enzymatic activity towards the basic sites of DNA [11].

Accumulating evidence has demonstrated that the heterogeneity of APE1/Ref-1 expression patterns is linked to different pathological conditions ranging from metabolic to differentiative disorders, including cancer and neurodegenerative diseases. Different kinds of human tumors are characterized by alterations in the subcellular distribution of APE1/Ref-1 with respect non-tumoral tissue [4,12]. The expression status of APE1/Ref-1 is altered in numerous cancers including prostate, lung, colon, and ovarian tumors. In addition, alterations in APE1/Ref-1 expression and mutations in the APE1/Ref-1 gene have been detected in patients with a variety of neurodegenerative diseases [2,13–18]. However, the pathophysiological roles of APE1/Ref-1 in kidney disease remain unclear.

In the present study, we evaluated whether APE1/Ref-1 was involved in ischemia-reperfusion (I/R)-induced kidney injury and whether APE1/Ref-1 played roles in H$_2$O$_2$-treated human proximal epithelial tubule (HK-2) cells. We also investigated whether APE1/Ref-1 overexpression exhibited cytoprotective or cytotoxic effects against H$_2$O$_2$-mediated apoptosis.

Methods

Animal experiments

The animal experiments were approved by the Animal Care Regulations Committee of the Chonnam National University Medical School (No. CNUH IACUC-18010), and the protocols conformed to the institutional guidelines for experimental animal care and use. Eight-week-old male C57BL6 mice were purchased from Samtako. Kidney I/R injuries were established by clamping both renal arteries for 20 minutes followed by 2 days of reperfusion. During the period of ischemia, body temperature was maintained by placing the rats on a 37 °C heating pad. Following removal of the clamps, the kidneys were inspected for 1 minute for the restoration of blood flow, as noted by a return to their original color, prior to closure of the abdomen. Sham-operated mice received identical surgical procedures with the exception of the microaneurysm clamps. The mice were sacrificed 2 days after reperfusion, blood was collected, and kidney tissue was divided for subsequent protein extraction.

Cell culture and reagents

HK-2 cells were passaged every 3–4 days in 100-mm dishes containing Dulbecco’s modified Eagle’s medium/F-12 medium supplemented with 10% fetal bovine serum (FBS), 100-U/mL penicillin, and 100-μg/mL streptomycin (Sigma). When the cells reached 70% to 80% confluence, they were detached with TrypLE Express (Invitrogen), centrifuged at 250 ×g for 3 minutes, replated, and maintained at 37 °C in an incubator containing 5% CO$_2$ atmosphere. For experimental use, the HK-2 cells were plated in 60-mm dishes in medium containing 10% FBS for 24 hours and then treated with H$_2$O$_2$. 

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Histologic analysis

Kidney tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 4 μm-thick sections. To assess histological morphology, immunohistochemical staining was performed using indicated antibodies and horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G secondary antibodies (Dako). The stained sections were imaged with a Nikon Eclipse Ni-U microscope. The quantitative analysis of the stained sections was performed using imageJ software (National Institutes of Health).

Confocal laser microscopy

Left kidneys of I/R and control mice were collected for immunofluorescence analyses. Tissue samples were prepared through deparaffination and hydration, and the cells fixed in 4% paraformaldehyde were prepared and blocked at room temperature for 2 hours. Rabbit or mouse monoclonal antibodies against Lotus tetragonolobus lectin (Vector Laboratories), calbindin-D28K (Invitrogen), Tamm-Horsfall protein (AbD serotec), and aquaporin-2 (Santa Cruz Biotechnology) were diluted 1:100 in blocking buffer and applied at 4 °C for 24 hours. After washing, the secondary antibody was diluted 1:200 in blocking buffer and applied at room temperature for 2 hours. After washing, coverslips were mounted onto microslides using a ProLong Gold Antifade Reagent with DAPI (Life Technologies).

Protein extraction

The kidneys were homogenized in ice-cold isolation solution containing 0.3-M sucrose, 25-mM imidazole, 1-mM ethylenediaminetetraacetic acid (EDTA), 8.5-μM leupeptin, and 1-mM phenylmethylsulfonyl fluoride (PMSF; pH 7.2). The homogenates were centrifuged and the total protein concentration was measured using a Pierce BCA protein assay kit (Thermo Fisher Scientific). All the samples were adjusted with isolation solution to normalize protein concentration and stored at −20 °C. HK-2 cells were harvested, washed twice with ice-cold phosphate-buffered saline (PBS), resuspended in lysis buffer (20-mM Tris-HCl [pH 7.4], 0.01-mM EDTA, 150-mM NaCl, 1-mM PMSF, 1-μg/mL leupeptin, and 1-mM Na3VO4), and briefly sonicated. After centrifugation, the supernatants were prepared as protein extracts and the protein concentrations were measured (Pierce BCA protein assay reagent kit).

Western blot analysis

Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 9% or 12% gels. The proteins were electrophoretically transferred onto nitrocellulose membranes using a Bio-Rad Mini Protein II apparatus (Bio-Rad). The blots were blocked with 5% milk in PBS-T (80-mM Na2HPO4, 20-mM NaH2PO4, 100-mM NaCl, and 0.1% Tween-20 [pH 7.5]) for 2 hours. Anti-Bcl-2 (3498), anti-Bax (2772), anti-cleaved caspase 3 (9661), anti-phospho extracellular signal-regulated kinase (p-ERK) (9101), anti-phospho-p38 mitogen-activated protein kinase (p-p38 MAPK) (4631), and anti-phospho JNK (p-JNK) (9251) antibodies (Cell Signaling Technology) were used. Phospho-NF-κB p65 (sc-33020; Santa Cruz Biotechnology), anti-NF-κB p65 (8242; Cell Signaling Technology), anti-ΙκBα (SC-1643; Santa Cruz Biotechnology), and anti-β-actin (a5316; Sigma) antibodies were diluted in blocking buffer and incubated with the blots overnight at 4 °C. The blots were then washed and incubated with peroxidase-conjugated secondary antibody (1:3,000). The selected bands were scanned (GS-700 Imaging Densitometry; Bio-Rad) and the density was determined (Molecular Analyst version 1.5; Bio-Rad).

Stable cell lines

To generate APE1/Ref-1 overexpressing cell lines, HK-2 cells were transfected with 2 μg of empty vector (Mock) or APE1/Ref-1 DNA using 6 μL of FuGENE HD reagent (Promega) in antibiotic-free DMEM-F12. Starting 1 day after transfection, transfectants were selected in DMEM-F12 containing 800-μg/mL zeocin which was refreshed every 3 days for 2 weeks. Colonies surviving in the selection medium were collected and sequentially plated in 48-, 12-, and 6-well plates and then in 60- and 100-mm dishes. Total cellular extracts were analyzed for APE1/Ref-1 expression by immunoblotting, thus revealing the expression of the ectopic flagged forms of wild type and the mutant form of the protein.
Small interfering RNA knockdown of APE1/Ref-1

RNA interference of APE1/Ref-1 was performed using an APE1/Ref-1–specific small interfering RNA (siRNA) from Ambion’s siRNA Target Finder Program: Ape1/Ref-1 siRNA (534 bp from the ATG), 5′-GUCUGGUACGACUGGAGUAtt-3′ (sense), and 5′-UACUCC AGUCGUACCAGCtt-3′ (anti-sense). siRNAs were prepared using a transcription-based method with a Silencer siRNA construction kit (Ambion).

Apoptosis assay

The number of apoptotic cells was quantified using the Ezway Annexin V-FITC apoptosis detection kit (KOMA Bio-tech) according to the manufacturer’s protocol. Cells were stained with Annexin V-FITC dye. Fluorescent intensity was measured by FACSCalibur flow cytometry (BD Biosciences).

Promotor activity of NF-κB

The transcriptional regulation of NF-κB was examined by the transient transfection of an NF-κB promoter-luciferase reporter construct (pGL3-NF-κB). HK-2 cells (5 × 10⁵) were seeded and grown until they reached 60% to 70% confluence, and pGL3-NF-κB wild-type and pGL3-empty were transfected into the cells using FuGene HD reagent according to the manufacturer’s protocol. The pRL-null plasmid encoding Renilla luciferase was included in all the samples to monitor transfection efficiency. At 24 hours after transfection, the levels of Firefly and Renilla luciferase activity were measured sequentially from a single sample using the Dual-Glo luciferase assay system (Promega). Firefly luciferase activity was normalized to Renilla activity and the relative amount of luciferase activity in the untreated cells.

Electrophoretic mobility shift assay

Nuclear extracts of the HK-2 cells were prepared with the NE-PER nuclear extraction reagent (Pierce Biotechnology). The biotin-labeled NF-κB oligonucleotide sequence was 5′-biotin-AGTTGAGGGGACTTTCCCAGGC-3′. The binding reactions contained 10 µg of the nuclear extract protein, buffer (10-mM Tris [pH 7.5], 50-mM KCl, 5-mM MgCl₂, 1-mM dithiothreitol, 0.05% Nonidet P-40, and 2.5% glycerol), 1 µg of poly (dl-dC), and 2-nM biotin-labeled DNA. The reactions were incubated at 23 °C for 20 minutes. The competition reactions were performed by adding 10-fold excess unlabeled double-stranded NF-κB consensus oligonucleotides to the reaction mixture. The reactions were electrophoresed on a 6% precasted Tris-borate-EDTA gel (Invitrogen) at 100 V for 1 hour 30 minutes in a 100-mM Tris-borate-EDTA buffer. The reactions were then transferred to a nylon membrane. The biotin-labeled DNA was detected with a LightShift chemiluminescent electrophoretic mobility shift assay kit (Pierce Biotechnology).

Statistical analysis

The results are expressed as the mean ± standard error of mean. Multiple comparisons among the three groups were performed using one-way analysis of variance and post hoc Tukey honestly significant difference tests. Differences with the p-values of <0.05 were considered significant.

Results

APE1/Ref-1 expression in in vivo ischemia-reperfusion–induced mouse kidney injuries

Fig. 1 shows the expression pattern of APE1/Ref-1 according to mouse I/R injury. The fluorescence of APE1/Ref-1 expression was highest in the cortex, modest in the outer medulla, and lowest in the inner medulla (Fig. 1A). The expression of APE1/Ref-1 that significantly increased after I/R injury compared to the control was observed by immunofluorescence (Fig. 1B). Fig. 1C shows the observation of the expression of APE1/Ref-1 in each part of the kidney by immunofluorescence. APE1/Ref-1 exhibited high expression in the proximal tubule, but weak expression was observed in the thick ascending limb and collecting duct in the mouse kidney (Fig. 1C).

Serum blood urea nitrogen and creatinine levels were significantly increased in I/R injured mice compared to the sham-operated controls (Fig. 2A). Consistent with this finding, the protein expression of APE1/Ref-1 increased after I/R injury (Fig. 2B). In addition, the protein expression levels of Bax and cleaved caspase 3 were increased, whereas Bcl-2 expression was decreased. We observed kidney...
tissue damage caused by I/R through H&E and PAS staining. There was a markedly increased kidney tubule-interstitial injury tissue in the I/R model compared to the control (Fig. 2C). Immunohistochemistry was performed to determine whether I/R-induced renal tissue damage was associated with the expression of APE1/Ref-1. Compared with the control, it was observed that the expression of APE1/Ref-1 increased in the I/R injury model (Fig. 2D). These results demonstrate that APE1/Ref-1 expression and apoptosis are induced by I/R kidney tissue damage.
Figure 2. Increased APE1/Ref-1 and apoptotic markers in mice with I/R injuries after 2 days. (A) Mice with renal I/R injury exhibited significantly higher serum creatinine (Scr) and blood urea nitrogen (BUN) levels compared to untreated I/R injury controls. (B) Western blotting analysis showed that the expression levels of apoptotic markers, such as pro-/anti-apoptotic Bax/Bcl-2, and cleaved caspase 3 (C-caspase 3) was altered and APE1/Ref-1 were increased in the I/R injury mouse model compared with those in the sham. (C) Immunohistochemical (IHC) staining of hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) in the kidneys of the control and I/R mice. Scale bar, 100 μm. Original magnification, ×200. (D) IHC staining of APE/Ref-1 in the kidneys of the control and I/R mice. Scale bar, 100 μm. Original magnification, ×200.

APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; Bax, Bcl-2–associated protein X; Bcl-2, B-cell lymphoma 2; I/R, ischemia-reperfusion.

Columns, means of three independent cases; bars, standard deviation.

*p < 0.05, I/R injury vs. sham.

Aggravation of H$_2$O$_2$-mediated injury by APE1/Ref-1 over-expression in HK-2 cells in vitro

Next, we investigated whether H$_2$O$_2$ treatment increased the expression of APE1/Ref-1 and apoptosis proteins in kidney proximal tubular HK-2 cells. Fig. 3A shows that 0-, 0.1-, 0.2-, 0.5-, and 1-mM H$_2$O$_2$ were treated in a concentration-dependent manner and harvested after 24 hours. APE1/Ref-1, Bax, and cleaved caspase-3 increased according to the concentration. Fig. 3B shows the time-de-
dependent observations of 0, 3, 6, 12, and 24 hours by treatment with 1-mM H$_2$O$_2$. The expression of APE1/Ref-1 and Bax was significantly increased after 24 hours. Next, we evaluated the effects of the overexpression of APE1/Ref-1 in H$_2$O$_2$-treated HK-2 cells in vitro. To determine the physiological effects of APE1/Ref-1, HK-2 cells were stably transfected with an empty vector (Mock) or a plasmid encoding human APE1/Ref-1. Stable cells were selected through the confirmation of the expression of zeocin, which was present in the backbone plasmid pCDNA4. The evaluation of Mock and APE1/Ref-1 stable cells following H$_2$O$_2$-mediated injury revealed decreased cell viability and increased the number of Annexin-V-positive cells in cells stably expressing APE1/Ref-1. Apoptotic cells were defined as Annexin V-FITC-positive cells. This result suggested that APE1/Ref-1 overexpression aggravated H$_2$O$_2$-mediated apoptotic and necrotic cell death (65.7% apoptosis in H$_2$O$_2$-treated HK-2 cells with APE1/Ref-1 overexpression versus 50.4% apoptosis in H$_2$O$_2$-treated HK-2 cells without APE1/Ref-1 overexpression) (Fig. 4).

**Figure 3.** H$_2$O$_2$-induced APE1/Ref-1 expression in HK-2 cells with various concentrations and exposure times of H$_2$O$_2$. (A) Exposure to H$_2$O$_2$ exhibited a dose-dependent increase in the protein expression of APE1/Ref-1, Bax, and cleaved caspase 3 (C-caspase 3). (B) The protein expression levels of APE1/Ref-1, Bax, and C-caspase 3 increased after H$_2$O$_2$ treatment in HK-2 cells. APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; Bax, B-cell lymphoma 2–associated protein X. Columns, means of three independent cases; bars, standard deviation. *p < 0.05 vs. H$_2$O$_2$-untreated cells.

Proapoptotic effects of APE1/Ref-1

To verify the apoptotic effects of APE1/Ref-1 in injured proximal tubule cells, the expression of proapoptotic proteins was evaluated in H$_2$O$_2$-treated Mock cells and cells stably expressing APE1/Ref-1. The expression level of the Bax/Bcl-2 ratio was increased in APE1/Ref-1 overexpressing cells (Fig. 5), and this effect was abolished by APE1/Ref-1 specific siRNA knockdown (Fig. 6).
Figure 4. Expression of apoptotic cells following APE1/Ref-1 overexpression. HK-2 cells and APE1/Ref-1 overexpressing HK-2 cells were treated with 1-mM H\textsubscript{2}O\textsubscript{2} for 6 hours and stained with Annexin V-FITC for 30 minutes, followed by flow cytometry analysis for the presence of apoptotic cells. APE1/Ref-1 overexpressing cells (65.7%) treated with H\textsubscript{2}O\textsubscript{2} exhibited increased apoptotic cells compared to H\textsubscript{2}O\textsubscript{2}-treated cells without overexpression (50.4%). The bar graph reveals apoptosis according to fluorescence values.

APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; SSC, side scatter.

Columns, means of three independent cases; bars, standard deviation.

*p < 0.05 vs. H\textsubscript{2}O\textsubscript{2}-untreated Mock cells; #p < 0.05 vs. H\textsubscript{2}O\textsubscript{2}-treated Mock cells.

Role of APE1/Ref-1 in mitogen-activated protein kinase signaling

Following treatment with 1-mM H\textsubscript{2}O\textsubscript{2}, the MAPK pathway components (e.g., extracellular signal-regulated kinase [ERK], c-Jun N-terminal kinase [JNK], and p38) were upregulated in APE1/Ref-1-overexpressing cells compared with Mock cells (Fig. 7A). The phosphorylation of p38, which is involved in the stabilization and mitochondrial accumulation of p38, was higher in H\textsubscript{2}O\textsubscript{2}-treated APE1/Ref-1 cells compared to Mock cells. Furthermore, the levels of activated p-ERK1/2 and phospho-JNK1/2 were enhanced in H\textsubscript{2}O\textsubscript{2}-treated APE1/Ref-1-overexpressing cells, and these effects were attenuated by transfection with siRNA targeting APE1/Ref-1 (Fig. 7B). Therefore, the involvement of the ERK/p38/JNK axis in apoptosis was associated with APE1/Ref-1 overexpression.

Effects of APE1/Ref-1 on NF-κB signaling

To further analyze the mechanisms through which APE1/Ref-1 was associated with the enhancement of apoptosis, we studied the expression of NF-κB, which is involved in the coordinated induction of genes that encode many stress-responsive and cytotoxic enzymes and related proteins. After treatment with 1-mM H\textsubscript{2}O\textsubscript{2}, NF-κB protein expression was observed in a 0, 1-, 2-, 4-, and 6-hour dependent manner. The expression of NF-κB protein was...
Figure 5. Enhancement of apoptotic protein expression induced by H$_2$O$_2$ in an APE1/Ref-1-overexpressing cell line. HK-2 cells transfected with APE1/Ref-1 exhibited increased apoptotic protein expression levels (Bax/Bcl-2 ratio) compared with Mock cells. APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; Bax, Bcl-2–associated protein X; Bcl-2, B-cell lymphoma 2.

significantly increased in APE1/Ref-1 overexpressing cells compared with Mock cells (Fig. 8A). Next, NF-κB expression by APE1/Ref-1 specific siRNA knockdown was observed. Cells were treated with 30 nM of scrambled siRNA and APE1/Ref-1 siRNA for 24 hours, and then treated with 1-mM H$_2$O$_2$ for 6 hours to obtain nuclear and cytoplasmic proteins, respectively, and NF-κB expression was observed. We observed the decreased expression of NF-κB nucleoproteins transfected with siRNA targeting APE1/Ref-1 (Fig. 8B). These results suggest that the expression of APE/Ref-1 and NF-κB is related to apoptosis.

Effects of APE1/Ref-1 on the transcriptional activation of NF-κB

NF-κB is an important transcription factor activating the expression of cyclooxygenase-2 and inducible nitric oxide synthase. Moreover, NF-κB is known to be activated by ROS. Therefore, we examined the role of APE1/Ref-1 in the H$_2$O$_2$-induced transcriptional activation of NF-κB. First, it was observed whether NF-κB phosphorylation increased in nucleoproteins after treatment with 1-mM H$_2$O$_2$. The expression of NF-κB proteins was observed to significantly increase the phosphorylation of NF-κB in APE1/Ref-1 overexpressing cells compared to Mock cells (Fig. 9A).
NF-κB is activated by a Cys-65 redox activation reaction that regulates several transcription factors present in the N-terminal region of APE1/Ref-1. We treated cells with an inhibitor of APE1/Ref-1 (E3330) and observed whether NF-κB activity was inhibited. NF-κB was significantly reduced by treatment with 100 μM E3330 (Fig. 9B). The promoter activity of NF-κB was increased following H₂O₂ exposure in HK-2 cells, and this increase was enhanced by APE1/Ref-1 transfection (Fig. 9C). Nuclear extracts from cells analyzed by electrophoretic mobility shift assays for activated NF-κB confirmed these findings (Fig. 9D). These results suggest that APE1/Ref-1 increased NF-κB pathway activity directly by activating the transcription factor NF-κB. Based on these results, a schematic diagram showing that APE1/Ref-1 induces apoptosis in association with MAPK and NF-κB signaling is presented in Fig. 10.

**Discussion**

Ischemic stress contributed to the upregulation of APE1/Ref-1 expression in the kidney. In HK-2 cells, oxidative stress induced increased APE1/Ref-1 expression, and APE1/Ref-1 aggravated H₂O₂-mediated apoptosis in APE1/Ref-1-overexpressing HK-2 cells. Moreover, the Bax/Bcl-

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**Figure 6. Downregulation of APE1/Ref-1 in H₂O₂-treated HK-2 cells.** HK-2 cells were harvested and equivalent amounts of protein were immunoblotted with anti-Bax, Bcl-2, and β-actin. RNA interference of APE1/Ref-1 was performed using an APE1/Ref-1-specific small interfering RNA (siRNA). The bar graph shows the relative protein expression of APE1/Ref-1 measured by densitometry. β-actin levels were analyzed as internal controls.

APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; Bax, Bcl-2–associated protein X; Bcl-2, B-cell lymphoma 2; siRNA, small interfering RNA; S-siRNA, scrambled siRNA; R-siRNA, Ref-1 siRNA.

Columns, means of three independent cases; bars, standard deviation.

*p < 0.05 vs. H₂O₂-untreated HK-2 cells; †p < 0.05 vs. H₂O₂-treated HK-2 cells in the absence of siRNA of APE1/Ref-1.
Figure 7. Effects of APE1/Ref-1 siRNA on the p-ERK1/2, p-p38, and p-JNK in HK-2 cells treated with \( \text{H}_2\text{O}_2 \). (A) Compared with Mock, APE1/Ref-1–overexpressing HK-2 cells exhibited increased protein expressions of p-ERK, p38, and p-JNK after pretreatment with \( \text{H}_2\text{O}_2 \) (1 mM). (B) Increased expression of p-ERK-1/2 and p-JNK was attenuated by siRNA of APE1/Ref-1, while p-p38 was not affected by APE1/Ref-1 overexpression.

APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; p-ERK, phosphorylation of extracellular signal-regulated kinase; p-JNK, phosphorylation of c-Jun N-terminal kinase; p-p38, phosphorylation of p38; siRNA, small interfering RNA; S-siRNA, scrambled siRNA; R-siRNA, Ref-1 siRNA.

\*p < 0.05 vs. \( \text{H}_2\text{O}_2 \)-untreated HK-2 cells, \#p < 0.05 vs. \( \text{H}_2\text{O}_2 \)-treated HK-2 cells in the absence of siRNA of APE1/Ref-1. Data are representative of at least three independent experiments.

2 ratio was increased in APE1/Ref-1–overexpressing cells but was decreased in HK-2 cells transfected with siRNA targeting APE1/Ref-1. We also demonstrated that APE1/Ref-1 was associated with the MAPK pathway and NF-\( \kappa \)B signaling. These findings suggest that the upregulation of APE1/Ref-1 is related to apoptosis after oxidative stress and that the inhibition of APE1/Ref-1 could have therapeutic potential in the management of acute kidney injuries.

APE1/Ref-1 is involved in the repair of DNA damage caused by oxidative stress [19] and has been shown to possess roles in the redox regulation of various transcription factors such as activator protein-1 [20], NF-\( \kappa \)B [21], early growth response-1 [22], NF-Y [23], HIF-1 [24], Myb [25], and p53 [26]. Moreover, this protein is involved in controlling cell-cycle arrest and apoptotic programs, with different functions in various contexts. Notably, the overexpression of APE1/Ref-1 confers resistance to apoptosis induced by chemotherapeutic drugs, radiation, hypoxia, and tumor necrosis factor (TNF) [27]. However, studies have also shown that APE1/Ref-1 is a potent signaling molecule in hyperacetylated breast cancer cells and promotes apoptosis-induced cell death [28]. In addition, \( \text{H}_2\text{O}_2 \) and/or hydroxyl radicals efficiently and rapidly promote a transient increase in APE1/Ref-1 protein levels, and various transcription factors are involved in the inducible expression of APE1/Ref-1 [18].

The regulatory functions modulating the activity of APE1/Ref-1 are complex and controlled through three
Figure 8. Effects of siRNA of APE1/Ref-1 on the expression of nuclear NF-κB p65 subunit proteins in HK-2 cells treated with H$_2$O$_2$. (A) Time-dependent NF-κB p65 expression levels were observed in Mock and APE/Ref-1 overexpressing cells after treatment with 1 mM H$_2$O$_2$. In the Mock cells, NF-κB p65 expression increased from 4 hours after H$_2$O$_2$ treatment, but it was observed that NF-κB p65 expression was highly activated in APE/Ref-1 overexpressing cells. (B) The H$_2$O$_2$-induced increase in the expression of the NF-κB p65 subunit in nuclear extracts of HK-2 cells was attenuated by siRNA of APE1/Ref-1.

APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; IκB, I kappa B; NF-κB, nuclear factor kappa B; siRNA, small interfering RNA; S-siRNA, scrambled siRNA; R-siRNA, Ref-1 siRNA.

Bars, standard deviation. Data are representative of at least three independent experiments.

*p < 0.05 vs. H$_2$O$_2$-untreated HK-2 cells, #p < 0.05 vs. H$_2$O$_2$-treated HK-2 cells in the absence of siRNA of APE1/Ref-1.

different mechanisms [29,30]: 1) increase in APE1/Ref-1 expression after transcriptional activation; 2) re-localization of APE1/Ref-1 from the cytoplasm to the nucleus; and 3) modulation of APE1/Ref-1 posttranslational modifications such as acetylation and phosphorylation. Selenomethionine, a posttranslational modification, is another mechanism through which p53 is stimulated by an APE1/Ref-1-dependent redox mechanism [26].

Oxidative DNA damage induces apoptosis and is involved in the activation of stress response pathways, including JNK and p38 MAPK pathways [31–33]. Previous studies examining the associations of APE1/Ref-1 and MAPKs have demonstrated that these proteins have protective effects against ROS owing to the redox function of APE1/Ref-1 [34,35]. Therefore, we also hypothesized that increases in APE1/Ref-1 by H$_2$O$_2$-induced ROS may be a stress response to modulate the adaptation to oxidative stress. However, when APE1/Ref-1 was knocked down using siRNA, the activation of JNK and p38 decreased. The current data also revealed the transcriptional activation of NF-κB in APE1/Ref-1-overexpressing cells using promotor assays. These conclusions somewhat differed from those of most studies on APE1/Ref-1, which have evaluated cancer cells or other organs with abnormal cell signaling and survival. Moreover, these discrepancies may also be related to the use of artificial overexpression rather than the natural internal expression by ROS. Alternatively, these results may also be related to the complexity of APE1/Ref-1 signaling,
Figure 9. Effects of APE1/Ref-1 on H$_2$O$_2$-induced NF-κB activation in HK-2 cells. (A) To observe the activity of NF-κB, the p-NF-κB was observed by extracting nucleoproteins from H$_2$O$_2$ treatment and APE/Ref-1 overexpression cells. The p-NF-κB was significantly increased in cells overexpressing APE/Ref-1 treated with 1-mM H$_2$O$_2$. (B) The inhibition of NF-κB activity was observed by treatment with E3330, an inhibitor of APE/Ref-1. NF-κB was significantly reduced by treatment with 100 µM E3330. (C) The transcriptional regulation of NF-κB was examined by the transient transfection of an NF-κB promoter-luciferase reporter construct (pGL3-NF-κB). Firefly luciferase activity was normalized to Renilla activity and the relative amount of luciferase activity in the untreated cells. The promoter activity of NF-κB was increased following H$_2$O$_2$ exposure in HK-2 cells, and this increase was enhanced by APE1/Ref-1 transfection. (D) Electrophoresis mobility gel shift assay was used to observe whether the overexpression of H$_2$O$_2$-treated APE1/Ref-1 increased the binding of nucleoproteins to NF-κB. The expression of NF-κB was also increased in cells treated with 1 mM H$_2$O$_2$ and cells overexpressing APE1/Ref-1, but NF-κB transcription factor binding was further increased in cells overexpressing APE1/Ref-1 treated with H$_2$O$_2$. APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; NF-κB, nuclear factor kappa B; p-NF-κB, phosphorylation of NF-κB. Data are representative of at least three independent experiments.

*p < 0.05 vs. H$_2$O$_2$-untreated Mock cells; #p < 0.05 vs. H$_2$O$_2$-treated Mock cells.
which includes various mechanisms and interactions with other proteins and effector genes.

NF-κB is a major transcription factor involved in the synthesis of critical cell survival proteins in response to cellular stress [36,37]. Controversy regarding the dual roles of NF-κB in enhancing or inhibiting apoptosis has been difficult to reconcile [38]. In apoptosis, NF-κB is activated following TNF-α treatment in several cell lines [39]. Moreover, the presence of NF-κB binding sites in genes encoding interleukin-1β converting enzyme protease, c-myc, and TNF-α, which are all involved in apoptosis and cell death, has been demonstrated [1,40]. The current study revealed that H₂O₂ increased APE1/Ref-1 expression and apoptosis and was associated with the activation of transcription factor NF-κB.

In summary, the findings of this study demonstrated that the promoter activity of NF-κB was increased by H₂O₂ exposure in HK-2 cells and that this effect was blocked by siRNA targeting APE1/Ref-1. Furthermore, APE1/Ref-1 sequentially activated the ERK/p38/JNK axis in the apoptotic pathway. These findings demonstrated that APE1/Ref-1 suppression protected HK-2 cells from H₂O₂-induced tubular injury through the inhibition of the promoter activity of the NF-κB promoter. Therefore, APE1/Ref-1 inhibitors may represent novel therapeutic agents for the treatment of acute kidney injuries, such as I/R-induced kidney injury.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available on request from the corresponding author.

Figure 10. Schematic diagram of tubular injury by APE1/Ref-1 regulation. Overexpression and knockdown of APE1/Ref-1 in the I/R mouse model and in H₂O₂-treated HK-2 cells are associated with apoptosis regulation, suggesting that they act on renal tubular injuries. APE1/Ref-1, apurinic/apyrimidinic endonuclease 1/redox factor-1; Bax, Bcl-2–associated protein X; Bcl-2, B-cell lymphoma 2; ERK, extracellular signal-regulated kinase; I/R, ischemia-reperfusion; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor kappa B.
Authors’ contributions

Conceptualization: HYK, SWK, EHB
Data curation: HYK, JSP, HSC
Methodology: HYK, JSP
Formal analysis: CSK, SKM
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Resources: BHJ, SKM
Writing–original draft: HYK, EHB
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References


Risk of mortality and cause of death according to kidney function parameters: a nationwide observational study in Korea

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Background: Further study is warranted to determine the association between estimated glomerular filtration rate (eGFR) or albuminuria and the risk of death from diverse causes.

Methods: We screened >10 million general health screening examinees who received health examinations conducted in 2009 using the claims database of Korea. After the exclusion of those previously diagnosed with renal failure and those with missing data, 9,917,838 individuals with available baseline kidney function measurements were included. The primary outcome was mortality and cause-specific death between 2009 and 2019 identified through death certificates based on the diagnostic codes of International Classification of Diseases, 10th revision. Multivariable Cox regression analysis adjusted for various clinicodemographic and social characteristics was used to assess mortality risk.

Results: The hazard ratio of death was significantly high in both the eGFR <60 mL/min/1.73 m² and in the eGFR ≥120 mL/min/1.73 m² groups in univariable and multivariable regression analyses when compared to those within the reference range (eGFR of 90–120 mL/min/1.73 m²). The results were similar for death by cardiovascular, cancer, infection, endocrine, respiratory, and digestive causes. We also found that albuminuria was associated with higher risk of death regardless of eGFR range, and those in the higher categories of dipstick albuminuria showed higher risk.

Conclusion: We reconfirmed the significant association between eGFR, albuminuria, and mortality. Healthcare providers should keep in mind that albuminuria and decreased eGFR as well as kidney hyperfiltration are independent predictors of mortality.

Keywords: Albuminuria, Epidemiology, Glomerular filtration rate, Glomerular hyperfiltration, Kidney function test
Introduction

The kidney is a vital organ for various health functions. Impairment in kidney function is related to a higher risk of adverse medical outcomes and mortality. Chronic kidney disease (CKD), a state of kidney function impairment, is associated with a substantial socioeconomic burden; thus, there is considerable societal interest in the consequences and risk factors related to kidney dysfunction [1]. Representative kidney function parameters, namely, estimated glomerular filtration rate (eGFR) and albuminuria, are measured to screen and assess kidney health [2,3].

The association between kidney dysfunction and mortality is complex, although the presence of this linkage is well established [4]. Previous studies have highlighted the clinical significance of the state of supranormal estimated glomerular, and the association between eGFR and death risk has been reported to be U-shaped [4–6]. In addition, previous studies have reported the clinical significance of albuminuria in the general population, and quantified albuminuria showed a linear association with mortality [4]. However, as mortality occurs due to various causes, additional study is warranted to investigate the association between kidney function parameters and death by specific causes. Such an investigation is important, as such a U-shaped association between eGFR and mortality may only represent death from cardiovascular causes and not noncardiovascular death such as cancer- or infection-related death [7]. Additionally, whether albuminuria has clinical significance for diverse causes of death needs to be studied, as urine albumin is mostly considered to be a biomarker related to cardiometabolic disorders. This information provides evidence for the clinical importance of early screening and risk stratification based on kidney function parameters in the general population.

In this study, we examined the association between eGFR or albuminuria and the risk of diverse causes of death in a large-scale health screening database in Korea. We hypothesized that the association between kidney function parameters and mortality risk would be present for various causes of death.

Methods

Ethical considerations

This study was approved by the Institutional Review Board of Seoul National University Hospital (No. E-2111-037-1270). The use of the National Health Insurance Database (NHID) was approved by a government organization. The study was conducted in accordance with the Declaration of Helsinki.

Study setting

In Korea, National Health Insurance is provided to all citizens of the Republic of Korea through the National Health Insurance Service. The NHID provided by the National Health Insurance Service is an insurance claims database and contains information on sociodemographic variables, national general health screening, and mortality. As enrollment in the health insurance system is mandatory, the database enabled us to study nationwide medical information, as done in our previous studies [8–10].

Study population

We aimed to investigate the association between kidney function parameters and mortality risk in the general population of Korea, we screened 10,585,843 adults (aged ≥20 years) examinees who underwent a national health screening conducted in 2009. We excluded 1) 9,602 individuals previously diagnosed with end-stage kidney disease, because kidney function parameters are different in those who have undergone dialysis or transplantation, and 2) 658,403 individuals with missing data. In the final study dataset, 9,917,838 individuals with available baseline kidney function measurements were included (Fig. 1).

Study exposures

The study exposures were eGFR and dipstick albuminuria, which were measured in national health screenings. As the main exposure category, eGFR values were grouped as <60, 60–90, 90–120, and ≥120 mL/min/1.73 m². eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) equation based on creatinine values measured by
the Jaffe method. Albuminuria categories were defined as negative/trace, 1+, 2+, and 3+/4+ values.

Data collection

The NHID provided the baseline characteristics, including age, sex, low-income status, history of smoking, drinking, physical activity, history of diabetes mellitus, hypertension, body mass index, blood pressure, and baseline laboratory parameters, including hemoglobin levels, fasting glucose values, and lipid profiles, of the study subjects. Low-income status was defined as income below the 20th percentile of the country. History of underlying diabetes mellitus, hypertension, and hyperlipidemia was inferred from the diagnostic codes of International Classification of Diseases, 10th revision (ICD-10) and the prescribing history of related medications. The specific ICD-10 codes used for comorbidities were as follows: for diabetes, ICD-10 codes of E10 (type 1 diabetes mellitus), E11 (type 2 diabetes mellitus), E12 (Malnutrition-related diabetes mellitus), E13 (Other specified diabetes mellitus), and E14 (unspecified diabetes mellitus); and for dyslipidemia, ICD-10 code of E78 (disorders of lipoprotein metabolism and other lipemias). Lastly, ICD-10 codes for hypertension were I10 (essential hypertension), I11 (hypertensive heart disease), I12 (hypertensive renal disease), I13 (hypertensive heart and renal disease), and I14 (secondary hypertension). A heavy alcohol drinker was defined as an individual consuming 0–30 g of alcohol per day. Regular physical activity was defined as moderate-intensity physical activity ≥5 days or vigorous-intensity physical activity ≥3 days per week. Information related to physical activity degree was collected through a questionnaire used in national health screenings [11].

Study outcomes

The primary outcome was the cause of death, which was identified through death certificates. The cause of death was classified as the primary cause of death based on ICD-10 diagnostic codes (Supplementary Table 1, available online). Study follow-up was performed until December 31, 2019.

Statistical analysis

We expressed categorical variables as numbers (percentages) and continuous variables as the mean (± standard deviation). We performed Cox regression analysis to investigate the association between kidney function exposures and risk of death by specific causes. To handle potential confounding effects, we constructed multivariable regression models. The full model was adjusted for age, sex, smoking, alcohol consumption, physical activity, body mass index, systolic blood pressure, history of diabetes mellitus, hypertension, hyperlipidemia, fasting glucose, and total cholesterol. In addition, in the multivariable model assessing eGFR as the exposure variable, dipstick albuminuria was included as an additional adjustment variable, and eGFR was included in the adjusted variables when albuminuria was assessed as the exposure variable. Statistical significance was asserted at a two-sided p-value of <0.05. Statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc.).

Results

Baseline characteristics

A comparison of baseline characteristics between eGFR groups is presented in Table 1. The higher eGFR group had a greater proportion of individuals of younger age and male sex. The group with a higher eGFR tended to have a
Table 1. Baseline characteristics of the study groups according to eGFR

<table>
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<tr>
<th>Variable</th>
<th>&lt;60  (n = 684,485)</th>
<th>60–90 (n = 5,318,506)</th>
<th>90–120 (n = 3,245,608)</th>
<th>≥120 (n = 669,239)</th>
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<td>Age (yr)</td>
<td>55.37 ± 15.74</td>
<td>48.82 ± 13.68</td>
<td>43.86 ± 12.96</td>
<td>42.80 ± 14.52</td>
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<td>Male sex</td>
<td>330,305 (48.3)</td>
<td>2,964,619 (55.7)</td>
<td>1,768,223 (54.5)</td>
<td>357,571 (53.4)</td>
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<td><strong>Body shape measures</strong></td>
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<tr>
<td>Height (cm)</td>
<td>161.53 ± 9.73</td>
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<td>Weight (kg)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Weight circumference (cm)</td>
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<td>Smoking history</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>448,677 (65.6)</td>
<td>3,156,232 (59.3)</td>
<td>1,903,600 (58.7)</td>
<td>396,622 (59.3)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>106,769 (15.6)</td>
<td>820,191 (15.4)</td>
<td>418,109 (12.9)</td>
<td>81,093 (12.1)</td>
</tr>
<tr>
<td>Current-smoker</td>
<td>129,039 (18.9)</td>
<td>1,342,083 (25.2)</td>
<td>923,899 (28.5)</td>
<td>191,524 (28.6)</td>
</tr>
<tr>
<td>Drinkers*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinker</td>
<td>421,893 (61.6)</td>
<td>2,797,673 (52.6)</td>
<td>1,583,268 (48.8)</td>
<td>322,519 (48.2)</td>
</tr>
<tr>
<td>Mild drinker</td>
<td>225,684 (33.0)</td>
<td>2,115,553 (39.8)</td>
<td>1,378,987 (42.5)</td>
<td>282,302 (42.2)</td>
</tr>
<tr>
<td>Heavy drinker</td>
<td>36,908 (5.4)</td>
<td>405,280 (7.6)</td>
<td>283,353 (8.7)</td>
<td>64,418 (9.6)</td>
</tr>
<tr>
<td>Regular physical activity²</td>
<td>133,339 (19.5)</td>
<td>1,001,108 (18.8)</td>
<td>544,318 (16.8)</td>
<td>101,900 (15.2)</td>
</tr>
<tr>
<td>Low income³</td>
<td>116,014 (17.0)</td>
<td>1,012,050 (18.0)</td>
<td>656,962 (20.2)</td>
<td>146,877 (22.0)</td>
</tr>
<tr>
<td><strong>Baseline comorbidities</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>109,829 (16.1)</td>
<td>474,051 (8.9)</td>
<td>235,131 (7.2)</td>
<td>54,636 (8.2)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>292,029 (42.7)</td>
<td>1,463,229 (27.5)</td>
<td>677,855 (20.9)</td>
<td>142,732 (21.3)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>192,190 (28.1)</td>
<td>1,043,428 (19.6)</td>
<td>473,998 (14.6)</td>
<td>90,963 (13.6)</td>
</tr>
<tr>
<td><strong>Laboratory measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.39 ± 16.01</td>
<td>122.88 ± 15.09</td>
<td>121.45 ± 14.73</td>
<td>121.29 ± 15.05</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.19 ± 10.19</td>
<td>76.64 ± 10.03</td>
<td>75.82 ± 10.04</td>
<td>75.40 ± 10.24</td>
</tr>
<tr>
<td>Impaired fasting glucose (mg/dL)</td>
<td>102.00 ± 29.33</td>
<td>97.74 ± 23.5</td>
<td>96.01 ± 23.02</td>
<td>95.87 ± 24.99</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.67 ± 1.68</td>
<td>14.00 ± 1.59</td>
<td>13.95 ± 1.60</td>
<td>13.80 ± 1.60</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>198.98 ± 43.92</td>
<td>197.73 ± 41.32</td>
<td>191.99 ± 40.56</td>
<td>188.10 ± 42.56</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>61.37 ± 66.82</td>
<td>55.41 ± 25.42</td>
<td>56.36 ± 28.42</td>
<td>60.20 ± 48.09</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>118.64 ± 90.70</td>
<td>124.09 ± 224.17</td>
<td>119.89 ± 239.64</td>
<td>108.67 ± 108.34</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>37.27 ± 23.01</td>
<td>77.00 ± 7.65</td>
<td>100.75 ± 8.12</td>
<td>159.48 ± 139.62</td>
</tr>
<tr>
<td>Urine protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative, trace</td>
<td>643,468 (94.0)</td>
<td>5,187,036 (97.5)</td>
<td>3,181,300 (98.0)</td>
<td>655,242 (97.9)</td>
</tr>
<tr>
<td>Positive</td>
<td>41,017 (6.0)</td>
<td>131,470 (2.5)</td>
<td>64,308 (2.0)</td>
<td>13,997 (2.1)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation or number (%). eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*Drinkers are categorized into three groups: nondrinkers (0 g/day), mild drinkers (0–30 g/day), and heavy drinkers (≥30 g/day).

²Regular physical activity was defined as moderate-intensity physical activity ≥5 days or vigorous-intensity physical activity ≥3 days per week.

³Individuals included in the lowest quartile (regarding required insurance fees or receiving free insurance) were categorized as the low-income group.

lower body mass index and included a higher proportion of current smokers and heavy drinkers. In addition, the high eGFR group had a more favorable lipid profile.

On the other hand, the lower eGFR group had a higher proportion of individuals with underlying hypertension, diabetes, and hyperlipidemia. Impaired fasting glucose and cholesterol levels were higher and hemoglobin levels were lower in the group with low eGFR. In addition, the prevalence of albuminuria was more than two-fold in the group with eGFR <60 mL/min/1.73 m² as in other eGFR groups.
Risk of all-cause death and death by specific causes according to kidney function parameters

In the four eGFR categories, the incidence rates of death and the hazard ratios for the causes of death are shown in Table 2 and Fig. 2. In univariable regression analysis, hazard ratios were significantly higher in those with lower eGFR than in those with eGFR of 90–120 mL/min/1.73 m². In addition, the group with eGFR ≥120 mL/min/1.73 m² also showed a significantly higher risk of death. The results

<table>
<thead>
<tr>
<th>Causes of death</th>
<th>eGFR (mL/min/1.73 m²)</th>
<th>No. of specific diseases</th>
<th>No. of events</th>
<th>Incidence rate (/1,000 PY)</th>
<th>Univariable model</th>
<th>Multivariable model*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>All-cause</td>
<td>&lt;60</td>
<td>684,485</td>
<td>85,597</td>
<td>12.682</td>
<td>3.95 (3.91–3.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>277,125</td>
<td>5.135</td>
<td>1.60 (1.59–1.61)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>90–120</td>
<td>3,245,608</td>
<td>106,531</td>
<td>3.215</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>≥120</td>
<td>669,239</td>
<td>29,000</td>
<td>4.271</td>
<td>1.33 (1.31–1.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infection</td>
<td>&lt;60</td>
<td>684,485</td>
<td>2,499</td>
<td>0.370</td>
<td>5.17 (4.88–5.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>6,731</td>
<td>0.125</td>
<td>1.74 (1.66–1.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>90–120</td>
<td>3,245,608</td>
<td>2,377</td>
<td>0.072</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>≥120</td>
<td>669,239</td>
<td>711</td>
<td>0.105</td>
<td>1.46 (1.35–1.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malignancy</td>
<td>&lt;60</td>
<td>684,485</td>
<td>22,941</td>
<td>3.399</td>
<td>2.65 (2.61–2.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>104,304</td>
<td>1.933</td>
<td>1.50 (1.49–1.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>90–120</td>
<td>3,245,608</td>
<td>42,574</td>
<td>1.285</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>≥120</td>
<td>669,239</td>
<td>10,773</td>
<td>1.587</td>
<td>1.24 (1.21–1.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endocrine</td>
<td>&lt;60</td>
<td>684,485</td>
<td>4,952</td>
<td>0.734</td>
<td>10.65 (10.14–11.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>7,086</td>
<td>0.131</td>
<td>1.90 (1.82–2.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>90–120</td>
<td>3,245,608</td>
<td>2,286</td>
<td>0.069</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
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<td>≥120</td>
<td>669,239</td>
<td>765</td>
<td>0.113</td>
<td>1.64 (1.51–1.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>&lt;60</td>
<td>684,485</td>
<td>21,479</td>
<td>3.182</td>
<td>6.32 (6.19–6.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>52,763</td>
<td>0.978</td>
<td>1.94 (1.91–1.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>90–120</td>
<td>3,245,608</td>
<td>16,707</td>
<td>0.504</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
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<td>669,239</td>
<td>4,681</td>
<td>0.689</td>
<td>1.37 (1.33–1.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory</td>
<td>&lt;60</td>
<td>684,485</td>
<td>8,647</td>
<td>1.281</td>
<td>5.33 (5.17–5.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>25,986</td>
<td>0.481</td>
<td>2.00 (1.95–2.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>90–120</td>
<td>3,245,608</td>
<td>7,975</td>
<td>0.241</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>≥120</td>
<td>669,239</td>
<td>2,316</td>
<td>0.341</td>
<td>1.42 (1.36–1.49)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digestive</td>
<td>&lt;60</td>
<td>684,485</td>
<td>2,827</td>
<td>0.419</td>
<td>2.98 (2.85–3.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>60–90</td>
<td>5,318,506</td>
<td>8,920</td>
<td>0.165</td>
<td>1.16 (1.14–1.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>90–120</td>
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<td>4,657</td>
<td>0.141</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
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<td>≥120</td>
<td>669,239</td>
<td>1,693</td>
<td>0.249</td>
<td>1.78 (1.68–1.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal</td>
<td>&lt;60</td>
<td>684,485</td>
<td>3,878</td>
<td>0.575</td>
<td>22.77 (21.13–24.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>5,318,506</td>
<td>3,526</td>
<td>0.065</td>
<td>2.58 (2.39–2.78)</td>
<td>&lt;0.001</td>
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<tr>
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<td>90–120</td>
<td>3,245,608</td>
<td>839</td>
<td>0.025</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
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<tr>
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<td>≥120</td>
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<td>256</td>
<td>0.038</td>
<td>1.50 (1.30–1.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Others</td>
<td>&lt;60</td>
<td>684,485</td>
<td>18,374</td>
<td>2.722</td>
<td>3.10 (3.04–3.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>60–90</td>
<td>5,318,506</td>
<td>67,809</td>
<td>1.256</td>
<td>1.43 (1.41–1.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>90–120</td>
<td>3,245,608</td>
<td>29,116</td>
<td>0.879</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>≥120</td>
<td>1,311 (1.28–1.34)</td>
<td>&lt;0.001</td>
<td>1.16 (1.13–1.19)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; PY, person-year.

*The multivariable model was adjusted for age, sex, smoking, alcohol consumption, regular physical activity, history of diabetes mellitus, hypertension, dyslipidemia, presence of dipstick albuminuria, body mass index, systolic blood pressure, fasting glucose, and total cholesterol.

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were significant for death by diverse causes, including death by malignancy, infection, cardiovascular, endocrine, respiratory, renal, and gastrointestinal disorders. When regression models were adjusted for various clinicodemographic characteristics, the overall results remained the same, and eGFR showed a U-shaped association with mortality risk by diverse causes, except that the risk of death by endocrine disorders became nonsignificant on multivariable analysis. Additionally, nonlinear associations in multivariable analysis between eGFR and causes of death are shown in Fig. 3.

When we further divided cancer events into specific cancer types (Supplementary Table 2, available online), the population with eGFR ≥120 mL/min/1.73 m² showed a significantly higher risk of cancer-specific death from oral cancer, esophageal cancer, stomach cancer, colorectal cancer, and lung cancer. The risk of death from biliary or pancreatic cancer was also higher for those with supranormal eGFR ranges; however, these associations did not reach statistical significance. In addition, the hazard ratios were higher for the risk of death by renal cancer, bladder cancer, and cervical cancer in those with GFR <60 mL/min/1.73 m². Those with a lower GFR showed a higher risk of cancer-related death; thus, the overall association between eGFR and death by specific cancers showed a U-shaped association.

**Risk of all-cause death and death by specific causes according to baseline albuminuria**

When we included the albuminuria results as the exposure, albuminuria was significantly associated with all-cause death and death by various causes (Table 3). Endocrine- and renal-related death showed a prominent linear association with albuminuria.

Similar to the above findings, there was a significant association between albuminuria and death by specific cancers (Supplementary Table 3, available online). In particular, linear associations between albuminuria and death by specific cancers were observed in oral cancer,
Figure 3. Nonlinear associations in the multivariable analysis between eGFR and causes of death. In the nonlinear associations, the overall results remained the same, and a U-shaped association was prominent in the all-cause, infection, respiratory, digestive, and other groups.

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio.
<table>
<thead>
<tr>
<th>Type of death</th>
<th>Dipstick</th>
<th>HR (95% CI)</th>
<th>p-value</th>
<th>HR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td></td>
<td>1.00 (Reference)</td>
<td>&lt;0.001</td>
<td>1.00 (Reference)</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>1+</td>
<td>2.20 (2.16–2.23)</td>
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<td>1.40 (1.38–1.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>3.24 (3.18–3.31)</td>
<td>&lt;0.001</td>
<td>1.77 (1.74–1.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3, 4+</td>
<td>4.71 (4.57–4.86)</td>
<td>&lt;0.001</td>
<td>2.29 (2.22–2.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td>2.21 (2.01–2.44)</td>
<td>&lt;0.001</td>
<td>1.38 (1.25–1.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>3.08 (2.70–3.524)</td>
<td>&lt;0.001</td>
<td>1.65 (1.44–1.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3, 4+</td>
<td>5.14 (4.24–6.2)</td>
<td>&lt;0.001</td>
<td>2.44 (2.02–2.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malignancy</td>
<td></td>
<td>5.23 (4.92–5.56)</td>
<td>&lt;0.001</td>
<td>1.94 (1.82–2.06)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>4.01 (3.85–4.19)</td>
<td>&lt;0.001</td>
<td>1.94 (1.86–2.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3, 4+</td>
<td>6.01 (5.64–6.41)</td>
<td>&lt;0.001</td>
<td>3.40 (3.18–3.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td>2.67 (2.59–2.76)</td>
<td>&lt;0.001</td>
<td>1.55 (1.50–1.60)</td>
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</tr>
<tr>
<td></td>
<td>2+</td>
<td>4.01 (3.85–4.19)</td>
<td>&lt;0.001</td>
<td>1.94 (1.86–2.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>3, 4+</td>
<td>6.01 (5.64–6.41)</td>
<td>&lt;0.001</td>
<td>3.40 (3.18–3.63)</td>
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</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td>2.17 (2.06–2.28)</td>
<td>&lt;0.001</td>
<td>1.37 (1.30–1.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>2.76 (2.56–2.97)</td>
<td>&lt;0.001</td>
<td>1.54 (1.43–1.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td>2.39 (2.21–2.58)</td>
<td>&lt;0.001</td>
<td>1.46 (1.35–1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>3.75 (3.39–4.15)</td>
<td>&lt;0.001</td>
<td>1.94 (1.75–2.15)</td>
<td>&lt;0.001</td>
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<td>5.15 (4.4–6.03)</td>
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<td>2.37 (2.02–2.78)</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
<td>6.00 (5.54–6.69)</td>
<td>&lt;0.001</td>
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</table>

**Table 3.** Risk of all-cause death and death by specific causes according to baseline albuminuria

- The multivariable model was adjusted for age, sex, smoking, alcohol consumption, regular physical activity, history of diabetes mellitus, hypertension, dyslipidemia, presence of dipstick albuminuria, body mass index, systolic blood pressure, fasting glucose, and total cholesterol.

esophageal cancer, stomach cancer, colorectal cancer, liver cancer, biliary cancer, pancreatic cancer, lung cancer, renal cancer, bladder cancer, lymphoma, multiple myeloma, and prostate cancer. There were also several exceptions: leukemia, breast cancer, and cervical cancer showed an inverted U-shape according to albuminuria, and a U-shape was observed for ovarian cancer.
Risk of death by specific causes according to baseline estimated glomerular filtration rate and albuminuria

Fig. 4 shows the heatmap according to risk relationships for all-cause mortality. The risk of mortality was divided by eGFR according to the presence or absence of albuminuria, as shown in Table 4. Most causes of death showed a U-shaped association with eGFR regardless of the presence or absence of albuminuria. Namely, both eGFR ranges <60 and ≥120 mL/min/1.73 m² were associated with a higher risk of death by various causes. The only finding that varied was in the multivariable model of the risk of death by malignancy. In those without albuminuria, the risk of cancer death was higher in those with higher eGFR (eGFR of 90–120 or ≥120 mL/min/1.73 m²), while those with eGFR <60 or 60–90 mL/min/1.73 m² showed lower adjusted risk of cancer death.

Those with baseline albuminuria, regardless of eGFR, showed a significantly higher risk of all-cause mortality and death by diverse causes than those with eGFR in the reference range (90–120 mL/min/1.73 m²) without albuminuria.

Discussion

This study using a nationwide population-based database demonstrated an association between the risk of various causes of death and eGFR or albuminuria. We found that both lower and higher eGFR ranges were independently associated with mortality by diverse causes. The overall risk of death was higher in those with albuminuria and showed an additive impact on eGFR. Overall, the results demonstrated the clinical significance of kidney function parameters related to the risk of death by various causes.

A U-shaped association between the risk of mortality and eGFR was observed in previous studies [4]. Confirmation of the U-shaped association between eGFR and the risk of death showed that kidney hyperfiltration is associated not only with cardiovascular disease but also with multiple causes of mortality, including cancer-, infection-, endocrine-, digestive-, and respiratory disorder-associated death. Furthermore, the risk of death was high in the presence of albuminuria within all categories of mortality. The major strengths of our study are that 1) we enrolled one of the largest cohorts including measurements for

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Dipstick albuminuria</th>
<th>eGFR (mL/min/1.73 m²)</th>
<th>HR (95% CI)</th>
</tr>
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<td>All-cause mortality</td>
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<td>&lt;60</td>
<td>3.72 (3.69–3.76)</td>
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<tr>
<td></td>
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<td>60–90</td>
<td>1.59 (1.58–1.60)</td>
</tr>
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<td>90–120</td>
<td>1.00 (Reference)</td>
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<td>1.33 (1.31–1.34)</td>
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<tr>
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<td>Positive</td>
<td>&lt;60</td>
<td>9.49 (9.03–9.68)</td>
</tr>
<tr>
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<td></td>
<td>60–90</td>
<td>3.45 (3.39–3.51)</td>
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<td>2.16 (2.09–2.22)</td>
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<tr>
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<td>2.98 (2.82–3.15)</td>
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Figure 4. Risk of death by all-cause mortality according to baseline eGFR and albuminuria. The heatmap is colored based on risk, where a red color indicates higher risk, and a green color indicates lower risk. The reference group included those with a baseline eGFR of 90–120 mL/min/1.73 m² without dipstick albuminuria.

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio.

Multivariable model was adjusted for age, sex, smoking, alcohol consumption, regular physical activity, history of diabetes mellitus, hypertension, dyslipidemia, presence of dipstick albuminuria, body mass index, systolic blood pressure, fasting glucose, and total cholesterol.
Table 4. Risk of death by specific causes according to baseline eGFR and albuminuria

<table>
<thead>
<tr>
<th>Type of death</th>
<th>Dipstick albuminuria</th>
<th>eGFR (mL/min/1.73 m²)</th>
<th>HR (95% CI)</th>
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<td>Multivariable model*</td>
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(Continued to the next page)
Table 4. Continued

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<td>Others</td>
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<td>1.22 (1.20–1.24)</td>
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<td>2.59 (2.31–2.89)</td>
<td>1.68 (1.51–1.88)</td>
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</tbody>
</table>

CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio.

*The multivariable model was adjusted for age, sex, smoking, alcohol consumption, regular physical activity, history of diabetes mellitus, hypertension, dyslipidemia, presence of dipstick albuminuria, body mass index, systolic blood pressure, fasting glucose, and total cholesterol.

important kidney function parameters; 2) we performed complete follow-up using a nationwide death registry; and 3) we identified various causes of death based on death certificates. Based on our study results, clinicians should be aware of the clinical significance of kidney function parameters measured in general health screenings, which are closely associated with survival, including death by diverse causes.

The KDIGO (Kidney Disease: Improving Global Outcomes) guidelines provide a heatmap summarizing the association between kidney function parameters and prognosis [12]. However, the previous version did not include the clinical significance of a supranormal eGFR, frequently defined as ≥120 mL/min/1.73 m² or similar values, although the importance of kidney hyperfiltration has been reported repeatedly [7,13,14]. In recent reports, kidney hyperfiltration, determined by eGFR or by measured GFR values [7], was considered another state of kidney function impairment leading to a rapid decline in kidney function [15]. We used an eGFR interval of 30 mL/min/1.73 m² and found that those with eGFR ≥120 mL/min/1.73 m² showed higher mortality risk than those within the reference range. However, there is currently no precise definition of hyperfiltration, which should be established. Increased proximal tubular sodium-glucose reabsorption activity or hyperactivity of the renin-angiotensin-aldosterone system may be the cause of kidney hyperfiltration, which is related to a worse prognosis [16]. This is supported by the fact that medications such as angiotensin receptor blockers or sodium-glucose cotransporter inhibitors reduce kidney hyperfiltration and improve patient prognosis [17]. As our study emphasizes the clinical significance of supranormal eGFR in death by various causes, we believe that supranormal eGFR should be defined by clear thresholds in clinical guidelines so that clinicians can appropriately interpret this most widely assessed kidney function parameter. We suggest clinicians first evaluate related illnesses in individuals showing kidney hyperfiltration and intervene when
necessary (e.g., prescribe renin-angiotensin-aldosterone system blockers) based on the assessment findings.

The prognostic importance of albuminuria, which has been reported in various clinical conditions, was confirmed in our study [18–20]. Regardless of baseline eGFR values, those with positive dipstick albuminuria showed a higher risk of death, including by various causes. Clinicians should note the linear relationship between ordinal dipstick albuminuria results and the risk of most causes of death. This relationship emphasizes the clinical utility of the dipstick albuminuria tests used in general health screenings, despite it being a semi-quantitative method.

As hypotheses explaining the mechanism related to the associations between kidney function parameters and noncardiovascular death. First, impairment of kidney function may be closely linked to immune dysfunction, even from the early stages, and could hinder appropriate body responses to infectious or cancerous conditions. Second, kidney dysfunction can cause difficulty in optimally managing diverse diseases, as medication pharmacokinetics/dynamics are altered in the state of kidney function impairment. Third, kidney dysfunction is associated with disturbance in systemic neurohormonal responses and impairment in certain pathways that have helpful effects on body homeostasis. Although this study cannot prove the underlying mechanism of the identified associations of kidney function parameters with death by diverse causes, such pathways should be investigated in future research focusing on the association between kidney function impairment and noncardiovascular diseases.

This study has several limitations. First, there is a sensitive issue in that the MDRD eGFR equation and dipstick test are not accurate as the kidney function parameters. Namely, eGFR remains an estimated parameter, and creatinine levels are affected by non-kidney factors such as muscle mass or diet. The MDRD study equations underestimate measured GFR in the range of GFR ≥60 mL/min/1.73 m² in healthy individuals or overestimate measured GFR in individuals with reduced muscle mass. Overestimated GFR in individuals with reduced muscle mass may bias the identified association in supranormal eGFR ranges. Also, dipstick albuminuria is not the gold-standard method to quantify proteinuria, as the dipstick test has limited quantitative accuracy [21]. The remaining possibility of intercenter variation and intervisit variation should also be considered. Next, the study population was limited to a single nationality; thus, the generalizability of the results should be confirmed by future studies. Lastly, the studied population might have suffered from the healthy volunteer bias, as we screened all those who received general health screenings, and the proportion of those with overt eGFR reduction was small.

In conclusion, we reconfirmed the correlation between eGFR, albuminuria, and mortality. The association was significant even for death by various causes, further highlighting the clinical importance of kidney hyperfiltration. In addition, for the evaluation of the state of kidney function impairment, a standard grading system for supranormal GFR is needed in addition to conventional grading for CKD. Healthcare providers should keep in mind that albuminuria and decreased eGFR, as well as renal hyperfiltration, are independent predictors of mortality.

**Additional information**

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**Conflicts of interest**

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions

Conceptualization, Supervision: DKK, KH, SP
Data curation, Formal analysis, Methodology, Resources: KH
Investigation: SL, YK, SC, HH, DKK, KH
Project administration: DKK, SP
Validation: SL, YK, SC, HH, YCK, SSH, HL, JPL, KWJ, CSL, YSK, DKK
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All authors read and approved the final manuscript.

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References


A collaborative model between dialysis clinics and a hospital center improves the quality of vascular access care and intervention for hemodialysis patients

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Background: This study reports the outcomes of a collaborative program between dialysis clinics and a referral hospital, which consisted of clinical monitoring and supplementary routine surveillance, for improving the quality of vascular access care.

Methods: This retrospective observational study was performed at five dialysis clinics as part of a 2-year collaborative program (2019–2020) in conjunction with a hospital-based dialysis access management center. A total of 392 hemodialysis patients (arteriovenous fistula [AVF], n = 339 and arteriovenous graft [AVG], n = 53) were included. Outcome measures included the prognosis of vascular access, clinic satisfaction, and referral rate to the hospital.

Results: Increased vascular access flow was observed and critical flow events decreased from the first to the second year (AVF: 18.3% vs. 12.7%, p < 0.001; AVG: 26.2% vs. 20.1%, p = 0.30). There were fewer percutaneous transluminal angioplasty events in the AVG group (0.77 per person-year vs. 0.51 per person-year, p = 0.005). New AVF or AVG creation events also remained low. All dialysis clinics were satisfied with the program. The overall referral rate from the participating clinics increased (65.7% vs. 72.0%) during the study period independently of the physical distance between the dialysis clinic and the hospital.

Conclusion: The collaboration between dialysis clinics and a referral hospital for improving the quality of vascular access care was successful in this study, and the model can be used by other clinics and hospitals looking to improve care coordination in dialysis patients.

Keywords: Arteriovenous shunt, Graft occlusion, Renal dialysis, Surgical blood flow velocity, Ultrasonography, Vascular patency

Introduction

Globally, the prevalence of end-stage kidney disease (ESKD) is increasing [1], with Taiwan having the highest prevalence in the world. Also, in Taiwan, 3,317 patients per million people received dialysis in 2015, and the prevalence of dialysis, regardless of ESKD, was 3,679 patients per million in 2019. The incidence rate of dialysis has also increased from 331 per million people in 2000 to 529 per million people in 2019 [2,3].
Despite preventive guidelines and clinical measures, however, the maintenance of vascular access for hemodialysis remains a significant challenge. Restoring vascular access remains the leading medical procedure in patients undergoing maintenance hemodialysis [4,5]. Loss of vascular access patency for both arteriovenous fistula (AVF) and arteriovenous graft (AVG) is associated with inadequate dialysis, morbidity, and mortality [6–8]. Therefore, preventing complications might lower morbidity rates, improve quality of life, and reduce the cost of health care in the dialysis population. The primary patency rate reported in a meta-analysis consisting of 46 articles was 60% (95% CI, 56%–64%) among 4,111 AVF cases (13 studies/21 cohorts) at 1 year and 51% (95% CI, 44%–58%) among 2,694 AVF cases (seven studies/12 cohorts) at 2 years [9]. Findings from the Dialysis Outcomes and Practice Patterns Study reported the primary AVF patency rates for patients from Europe/Australia/New Zealand and Japan to be 51% and 72% at 1 year and each 40% to 60% at 2 years [10]. In a study using a national database in Taiwan, the primary patency rates, from creation of AVF to intervention, were 55.4% and 40.8% at 1 and 2 years, respectively, while the primary patency rates of AVG were 33.4% and 13.7% at 1 and 2 years [11].

Clinical practice guidelines for vascular access recommend increased use of autogenous AVFs and the prevention of access dysfunction with surveillance and preemptive intervention [12]. To improve patient quality of life and care, the 2006 Kidney Disease Outcome Quality Initiative (K/DOQI) has recommended either clinical monitoring or routine vascular access flow surveillance for early identification of potential vascular access problems, allowing for timely intervention of access dysfunction. Vascular interventions are recommended when vascular access flow becomes <600 mL/min in grafts, <400–500 mL/min in fistulae, decreasess by 25%, or decreases to <1,000 mL/min over a 4-month period [12]. However, no clear consensus has been reached regarding optimal surveillance to identify a failing access route [13]. Subsequently, the 2019 K/DOQI guideline placed less emphasis on routine surveillance due to insufficient evidence to make a recommendation for surveillance in addition to routine clinical monitoring [14].

From our experience, supplementary routine surveillance provides an objective assessment for actively identifying potential vascular access problems at an early stage in hospital settings [8,15]. However, whether this would also benefit dialysis clinics is not known. In general, in Taiwanese patients who attend dialysis clinics (as opposed to hospital dialysis centers) for hemodialysis have few comorbidities, are stable, and have stable or no severe medical conditions [3]. For this reason, these dialysis clinics usually do not have surveillance equipment or certified technic-ians to perform regular tracking of vascular access flow rate (Qa), and they are also not able to perform vascular intervention even when problems are detected with clinical monitoring. Collaborative networks to support dialysis clinics with resources from hospital dialysis access management centers are critical for timely identification and intervention of potential vascular access problems, such as stenosis and thrombosis, in hemodialysis patients [16]. Our hospital-based dialysis access management center (Dialysis Access Management Center in Shin Kong Wu Ho-Su Memorial Hospital [SKH]) began to cooperate with regional dialysis clinics in 2018 to implement vascular access clinical monitoring and supplementary surveillance programs. Services provided included evaluation protocols for potential vascular access risk identification, which, once detected, facilitated the timely referral of patients to the dialysis access management center for further evaluation and/or intervention if necessary.

The aim of this investigation was to examine the effect of this collaborative program involving supplementary routine surveillance on the prognosis of vascular access in dialysis clinics and satisfaction rates from the participating clinics. The goal was to bridge the current system deficiencies to improve quality of care, patient navigation, and interprofessional collaboration. It was hypothesized that this collaborative model could improve the quality of care for patients with dialysis through early evaluation of vascular access problems with supplementary surveillance and enhance the quality of care at dialysis clinics.

**Methods**

**Study design**

This retrospective observational study was performed at six non-hospital-based dialysis clinics as part of a collaborative program with a hospital-based dialysis access management center (Dialysis Access Management Center in SKH) between January 2019 and December 2020. In addi-
tion to clinical monitoring as per the 2019 K/DOQI clinical practice guidelines, the Qa was measured by ultrasound dilution. Vascular access flow surveillance was performed every 3 months during the study period. The incidence of preemptive intervention and vascular access failure due to thrombotic occlusive events within both periods (including needing to perform percutaneous transluminal angioplasty [PTA], new AVG creation, or AVG creation) was recorded to determine the effect of the access flow-based collaborative program. A total of 392 hemodialysis patients with vascular access (AVF, n = 339 and AVG, n = 53) were included.

A fistulogram to evaluate the need for vascular interventions was recommended to patients with clinical indicators suggestive of dysfunction. In the event of a critical flow problem detected by surveillance (e.g., low-vascular access flow or a substantial flow decline based on the following definition adopted from K/DOQI clinical practice guidelines for vascular access), clinical indicators suggestive of dysfunction also needed to be present before scheduling a fistulogram to further evaluate the need for vascular intervention.

Outcome parameters and definitions

The main outcome measures included prognosis of vascular access in clinic dialysis patients (i.e., vascular access flow, incidence of critical flow, vascular access reconstruction rate, and vascular access balloon dilatation rate), satisfaction of the dialysis clinic with the collaborative model, and the referral rate to the surveillance program provider (i.e., the hospital-based dialysis access management center).

The incidence of critical flow events was defined as: event ratio = number of critical flow events / number of flow records. Vascular access flow problem was defined as having critical Qa of <600 mL/min in gr afts and <500 mL/min in fistulae, or Qa declined by >25% and falls below 1,000 mL/min over a 3-month period (Fig. 1).

The collaborating dialysis clinics were asked to complete a 5-point Likert satisfaction survey at the end of both 2019 and 2020. The survey consisted of 14 questions grouped into four categories, as follows: 1) satisfaction with the instructions provided regarding the measurement procedure; 2) satisfaction with the recommended critical flow criteria; 3) satisfaction of intervention planning upon identification of a critical flow problem; and 4) willingness to continue with the collaboration program (Supplementary Table 1, available online).

Access flow measurements by ultrasound dilution technique

The access flow measurement technique involves reversing the access lines during dialysis and using the ultrasound dilution methodology, as introduced by Krivitski [17], to measure the resulting fraction of recirculated blood entering the dialyzer. Vascular access flow was measured by a certified technician. A dilution ultrasound exam was performed during dialysis on the same day, in the first hours of dialysis. Qa was calculated using the formula as follows: \( Qa = Qb \times \left(1 - \frac{R}{R}\right) \), where \( Qb \) is the extracorporeal pump pressure and \( R \) is the fraction of recirculated blood.

Statistical analysis

Nonparametric continuous variables are presented as median and interquartile range values and tested with the Mann-Whitney U test. Parametric continuous variables are presented as mean and standard deviation values and tested with the t test for normal distribution. Categorical variables are presented as counts and percentages and tested with the chi-square test. The significance level was set at two-sided p-value of <0.05. All statistical analyses were performed using SAS version 9.4 (Windows NT version; SAS Institute, Inc.).

Ethical considerations

This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of SKH Memorial Hospital (no. 20170921R). Informed consent was waived by the same committee in view of the retrospective nature of the study and the fact that all procedures being performed were part of routine care.

Results

Improved quality of patient care

A total of 392 patients from the five participating dialysis
clinics (clinics A–E) were enrolled into this study (including 339 patients with AVF and 53 patients with AVG). Baseline patient demographics are reported in Table 1. The demographics for individual clinics are reported in Supplementary Table 2 (available online). The median age was similar between groups (65 years for AVF patients vs. 67 years for AVG patients). There were statistically significant increases in the median systolic blood pressure and median diastolic blood pressure in the AVF group compared to the AVG group (p < 0.05). There was also a statistically significant greater proportion of women in the AVF group (p = 0.001).

The overall ratio of critical flow events significantly decreased from the first year to the second year of surveillance in the AVF group (18.3% vs. 12.7%, p < 0.001) (Table 2). A similar trend was demonstrated in critical flow events in

### Table 1. Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AVF</th>
<th>AVG</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>339</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>65.0 (59.0–73.0)</td>
<td>67.0 (61.0–77.0)</td>
<td>0.20*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>140 (123–155)</td>
<td>131 (118–146)</td>
<td>0.03**</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70 (61–80)</td>
<td>65 (59–75)</td>
<td>0.03**</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>158 (46.6)</td>
<td>38 (71.7)</td>
<td>0.001***</td>
</tr>
<tr>
<td>Male</td>
<td>181 (53.4)</td>
<td>15 (28.3)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as number only, median (interquartile range), or number (%).

AVF, arteriovenous fistula; AVG, arteriovenous graft; DBP, diastolic blood pressure; SBP, systolic blood pressure.

*Mann-Whitney U test; **chi-square test.

*Statistically significant, p < 0.05.
the AVG group (26.2% vs. 20.1%, p = 0.30). Increased vascular access flow from the first-year surveillance period to the second-year surveillance period was observed in both the AVF and AVG groups, respectively, although the difference was not significant (Table 2).

Clinical outcomes associated with participation in the collaborative program are shown in Table 2. The number of PTA events decreased in both groups over the 2-year surveillance period, with a significant decline observed in the AVG group (0.77 per person-year vs. 0.51 per person-year, p = 0.005). The number of new AVF or AVG creations was similar between groups and remained low over time.

**Clinician satisfaction**

Pooled results from the satisfaction with collaboration survey from the five participating non–hospital-based dialysis clinics are shown in Fig. 2. A total of 35 medical personnel, including attending physicians, nurses, and certified dialysis technicians, replied to the satisfaction survey. At least three surveys were obtained from each of the clinics. Overall, dialysis clinics were satisfied with the collaborative program. The satisfaction rate increased from the first year of surveillance to the second year in all domains, including in the guidelines given for measurement procedures, the recommended critical flow criteria, the arrangement of subsequent evaluations, and intervention upon identification of critical flow problems. The rate of willingness to continue with the collaboration program was 100% by the end of the second year.

### Table 2. The ratio of critical flow, vascular access flow, and clinical events after dialysis clinics joined the vascular surveillance program

<table>
<thead>
<tr>
<th>Variable</th>
<th>AVF (N = 339)</th>
<th>AVG (N = 53)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ratio of critical flow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>207/1,133 (18.3)</td>
<td>123/972 (12.7)</td>
<td>0.0004**</td>
</tr>
<tr>
<td>Clinic A</td>
<td>72/460 (15.7)</td>
<td>47/414 (11.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Clinic B</td>
<td>23/129 (17.8)</td>
<td>19/118 (16.1)</td>
<td>0.72</td>
</tr>
<tr>
<td>Clinic C</td>
<td>60/277 (21.7)</td>
<td>29/238 (12.2)</td>
<td>0.005**</td>
</tr>
<tr>
<td>Clinic D</td>
<td>18/116 (15.5)</td>
<td>10/101 (9.9)</td>
<td>0.22</td>
</tr>
<tr>
<td>Clinic E</td>
<td>34/151 (22.5)</td>
<td>18/101 (17.8)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Vascular access flow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>1,035.80 ± 496.76 (n = 1,133)</td>
<td>1,076.30 ± 513.65 (n = 972)</td>
<td>0.07</td>
</tr>
<tr>
<td>Clinic A</td>
<td>1,101.43 ± 484.50 (n = 460)</td>
<td>1,141.62 ± 545.87 (n = 414)</td>
<td>0.25</td>
</tr>
<tr>
<td>Clinic B</td>
<td>1,063.64 ± 441.91 (n = 129)</td>
<td>1,100.17 ± 507.73 (n = 118)</td>
<td>0.55</td>
</tr>
<tr>
<td>Clinic C</td>
<td>972.38 ± 474.54 (n = 277)</td>
<td>991.89 ± 423.28 (n = 238)</td>
<td>0.63</td>
</tr>
<tr>
<td>Clinic D</td>
<td>1,172.76 ± 636.84 (n = 116)</td>
<td>1,159.50 ± 579.97 (n = 101)</td>
<td>0.87</td>
</tr>
<tr>
<td>Clinic E</td>
<td>823.18 ± 413.19 (n = 151)</td>
<td>896.34 ± 440.00 (n = 101)</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Clinical event ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of PTAs</td>
<td>0.324 (n = 110)</td>
<td>0.316 (n = 107)</td>
<td>0.80</td>
</tr>
<tr>
<td>No. of new AVF creations</td>
<td>0.003 (n = 1)</td>
<td>0 (n = 0)</td>
<td>0.32</td>
</tr>
<tr>
<td>No. of new AVG creations</td>
<td>0.006 (n = 2)</td>
<td>0 (n = 0)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Data are expressed as number (%), mean ± standard deviation, or ratio only. Event ratio = number of critical flow events / number of records in 1 year. AVF, arteriovenous fistula; AVG, arteriovenous graft; N, number of participants; n, number of records; PTA, percutaneous transluminal angioplasty.

*Critical flow defined as access flow of <500 mL/min or decreases of 25% and <1,000 mL/min over a 3-month period in the AVF group or <600 mL/min or decreases of 25% and <1,000 mL/min over a 3-month period in the AVG group. **Probability difference test. *T test.

*1 Statistically significant in the comparison between 2019 and 2020, p < 0.05.
Figure 2. Comparison of satisfaction survey responses from clinics between 2019 and 2020. (A) The percentage of clinics reporting either satisfaction or strong satisfaction, by year. (B) The mean of the satisfaction survey score, by year.
Enhanced interprofessional collaboration

The overall hospital referral rate (SKH) from the five participating clinics increased from year 1 to year 2 (65.7% vs. 72.0%). The referral rate was not directly associated with the physical distance between the non–hospital-based dialysis clinics and the participating hospital (Fig. 3; Supplementary Table 3, available online).

Discussion

Vascular access is critical for patients undergoing dialysis. Failure to obtain vascular access is associated with morbidity, discomfort, inconvenience, and increased costs [18]. In this study, we reported outcomes following the implementation of an integrated collaborative model for care and treatment of vascular access issues in hemodialysis patients based on clinical monitoring and supplementary access flow surveillance. We demonstrated an overall increase in the Qa, a decline in critical vascular flow events, and a reduction in both the number of PTA interventions and new AVG and AVF creations. The strength of this program is its introduction of supplementary routine surveillance to dialysis clinics. To the best of our knowledge, this is the first study to report outcomes like these from this type of collaborative model.

The implementation of access surveillance in dialysis clinics aims to detect problems at an early stage. Unfortunately, this type of program is problematic for many dialysis clinics for several reasons, including a lack of surveillance equipment due to cost; a lack of certified technicians to perform regular tracking of vascular access; and an inability to perform vascular intervention at some local dialysis clinics, even when access flow problems are detected (such as in Taiwan). Ozgen and Ozcan [16], in reaction to findings from a study that examined facility characteristics, commented that most facilities are functioning technically inefficiently. Interestingly, technical efficiency was significantly associated with the type of ownership.

Our study demonstrated that, by providing detailed...
guidance on criteria for identifying critical vascular access problems and having medical center support for the early evaluation of potential vascular access dysfunction, the quality of vascular access was improved (thus achieving the standard recommended by KDOQI guidelines). We reported that the number of PTAs was significantly decreased in the AVG group, while the number of new AVF and AVG creation events was maintained at low levels during the collaboration period (0.006 per patient-year in the AVF group; 0.057 per patient-year in the AVG group).

In Taiwan, the hemodialysis quality guidelines recommend the vascular access reconstruction rate to be monitored every 6 months and to be lower than 1.1 times the average rate of the previous 3 years. The ratio of vascular access reconstruction, as indicated in an annual report of kidney disease, was 1 to 2 cases per 1,000 patient-months from 2010 to 2014 [19]. By the end of 2019, the upper limit of the inter-hospital reconstruction rate (defined as the total number of patients who need re-creation of AVF or AVG per 100 total patient-months) was ≤0.49, while the upper limit of fistula reconstruction was ≤0.13 in the same hospital [20]. If the reconstruction rate of medical facilities exceeds the upper limit, a portion of the hemodialysis reimbursement may be deducted by the National Health Insurance in Taiwan.

Team-based interprofessional collaboration is warranted because vascular access care is a multistep process with different providers involved at different levels of care [21]. Our collaborative model supports the team-based approach and demonstrated improved vascular access outcomes. By providing coordinated care that includes a dialysis access management specialty center, interprofessional communication was improved, which facilitated early identification of potential vascular access problems, such as stenosis and thrombosis, in hemodialysis patients [22-24]. As noted by Murea and Woo [22] in 2021, a fragmented approach to hemodialysis patient care has, unfortunately, been the norm. Going forward, dialysis clinics, which are usually isolated in health care settings, should shift toward a patient-tailored care approach. In our current study, the satisfaction of the collaborating dialysis clinics was demonstrated by the results of the satisfaction survey, and during the collaboration period, there was also an increase in referrals to SKH for vascular access evaluation and intervention, regardless of the distance needed to travel. This indicates that both patients and dialysis clinics have gained more trust with the collaborating hospital and that the collaborative model has provided a system of support to improve the quality of care, patient navigation, and interprofessional collaboration.

There are several limitations associated with this study. First, because patient medical histories and data about vascular access before collaboration were not available, the heterogeneity in the health status of the study group was not considered. Vascular access outcomes may be affected by comorbidities, dialysis duration, and cause of end-stage renal disease. Second, because all dialysis patients in the participating clinics joined our collaborative program at the same time, this study lacked a control group. Future prospective studies should be designed to compare vascular flow and critical events between participants with or without collaborative surveillance to rule out possible confounding factors. Third, this study did not investigate the cost-effectiveness of an access flow-based surveillance program; however, previous studies have demonstrated the cost-effectiveness of such a program. In their study, McCarley et al. [25] reported a reduction in thrombosis rates in both AVG and AVF patients, which improved patient comfort and health care costs, and Wijnen et al. [26] reported that quality improvement programs, based on periodic access flow measurements, reduced the number of acute vascular access failures due to thrombotic events and also significantly reduced health care costs in patients with AVG.

This study demonstrates the success of a collaborative model between dialysis clinics and a hospital center for improving the quality of vascular access care and intervention. The strength of this program is its introduction of supplementary routine surveillance to dialysis clinics, which can be used as a model for other hospitals and their management of dialysis patients with vascular access. Establishing an integrated collaborative model for vascular access care and treatment between dialysis clinics and referral hospitals can alleviate some of the current difficulties encountered by dialysis clinics when caring for patients with a vascular access route. Importantly, a satisfactory prognosis of the vascular access for dialysis patients can be maintained with a collaborative program. The establishment of trust between patients, dialysis clinics, and partner referral hospitals benefits all stakeholders and encourages
referrals to the partner hospitals. Pursuing this “triple win” situation between patients, local dialysis clinics, and partner specialist hospital centers should be considered in other regions.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available in the article and in its online supplementary material.

Authors’ contributions

Conception, Formal analysis, Investigation, Methodology: CKW, CHL
Funding acquisition: CHL
Supervision: All authors.
Writing-original draft: CKW
Writing-review & editing: All authors.
All authors read and approved the final manuscript.

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Circulatory endostatin level and risk of cardiovascular events in patients with end-stage renal disease on hemodialysis

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Background: Endostatin is released during extracellular matrix remodeling and is involved in the development of vascular pathology and cardiovascular (CV) disease. However, the role of circulating endostatin as a biomarker of vascular calcification and CV events in patients undergoing hemodialysis (HD) remains unclear.

Methods: A total of 372 patients undergoing HD were prospectively recruited. Plasma endostatin levels were measured at baseline, and their associations with circulating mineral bone disease (MBD) biomarkers and abdominal aortic vascular calcification scores were analyzed. The primary endpoint was defined as a composite of CV and cardiac events.

Results: Plasma levels of patients in endostatin tertile 3 were significantly associated with low-density lipoprotein cholesterol levels and predialysis systolic blood pressure in multivariate analysis. However, endostatin levels did not correlate with circulating MBD biomarkers or vascular calcification scores. Patients in endostatin tertile 3 had a significantly higher cumulative event rate for the composite of CV events (p = 0.006). Endostatin tertile 3 was also associated with an increased cumulative rate of cardiac events (p = 0.04). In multivariate Cox regression analyses, endostatin tertile 3 was associated with a 4.37-fold risk for composite CV events and a 3.88-fold risk for cardiac events after adjusting for multiple variables.

Conclusion: Higher circulating endostatin levels were independently associated with atherosclerotic risk factors but did not correlate with MBD markers or vascular calcification. Higher circulating endostatin levels were associated with a greater risk of composite CV events in patients undergoing HD, and endostatin is a biomarker that helps to determine the high risk of CV events.

Keywords: Cardiovascular diseases, Endostatins, Hemodialysis, Vascular calcification
Introduction

Cardiovascular disease (CVD) is the most common cause of death in patients with end-stage renal disease (ESRD) undergoing hemodialysis (HD) [1]. Prior studies have shown that both traditional and non-traditional risk factors are multifactorial in the pathogenesis of CVD in patients with ESRD. Moreover, it is difficult to predict the occurrence of cardiovascular (CV) events in these patients [2]. ESRD patients with CVD often show no or atypical symptoms, resulting in delayed diagnosis and failure to receive appropriate management of CVD. Therefore, several studies have suggested biomarkers that can predict the occurrence of CV events in these patients [3,4], and several ongoing investigations have identified biomarkers that could support the prediction of CVD occurrence.

Endostatin is a carboxyl-terminal fragment of type XVIII collagen, which is present in various endothelial and epithelial basement membranes [5]. It exerts anti-angiogenic and anti-fibrotic effects by inhibiting the proliferation and migration of endothelial cells [6]. Endostatin can be found in the circulatory system, and circulating serum endostatin levels have been suggested to be a marker of extracellular matrix breakdown in several CV pathologic conditions [7]. Previous studies have reported that circulating endostatin is a relevant biomarker that can improve risk prediction in various disease conditions, including CV events [8]. It has also been reported that circulating endostatin is associated with the progression of atherosclerosis and vascular calcification. Serum endostatin levels are increased in patients with coronary artery disease, and significant correlations between endostatin and coronary artery calcification or aortic valve calcification have been reported in prior studies [5,9].

Patients with ESRD have a high prevalence of vascular calcification, which shows distinct pathophysiological features compared to those in the general population [2,10]. Patients with ESRD undergoing HD are at risk of atherosclerotic injury because they are constantly exposed to endothelial injury, oxidative stress, and inflammation. Repeated exposure to hemodynamic stress during dialysis worsens this atherosclerotic vascular pathology [11]. Furthermore, these patients already have various traditional risk factors for CV events that trigger the formation of atherosclerotic plaques and intimal calcification [2,12]. Therefore, patients undergoing HD have a higher prevalence of intimal vascular calcification, which is an independent risk factor for CV events and mortality in these populations [13,14]. In addition, factors related to mineral bone diseases (MBDs) in chronic kidney disease (CKD), including hyperphosphatemia, hypercalcemia, and secondary hyperparathyroidism, can cause mineral deposition on the medial layer of the vascular wall, leading to medial vascular calcification [15]. Therefore, patients with ESRD tend to have both intimal and medial layer calcification, which further increases the risk of CV events.

Considering the function of endostatin and its association with vascular calcification, it can be assumed that endostatin might have a predictive role in the occurrence of vascular calcification and CV events in patients with ESRD on HD. There is a clear need to confirm the clinical relevance of endostatin in these patients, as few studies have analyzed the association among them. In this study, we measured circulating endostatin levels and investigated their association with CV events in ESRD patients undergoing HD. We also analyzed the relationship between endostatin levels and clinical characteristics as well as circulating MBD biomarkers.

Methods

Study population and design

Participants were recruited from the K-cohort, a multicenter, internet-based, prospective cohort of patients undergoing HD in South Korea. The K-cohort study was conducted to evaluate the morbidity and mortality of patients with ESRD on HD starting in 2016 (CRIS No. KCT0003281). Patients with ESRD aged >18 years on HD were enrolled in the K-cohort if they were undergoing HD three times a week for >3 months. The exclusion criteria were pregnancy, hematologic malignancy, active or invasive solid tumor, and life expectancy of <6 months. Clinical data, laboratory results, and blood samples were prospectively collected from patients with ESRD on HD at the baseline and follow-up visits. Patient outcomes, such as CV events, comorbidities, and mortality, were analyzed. Detailed cohort design and inclusion/exclusion criteria have been previously described [16,17].

A total of 381 patients on HD with whole-plasma sam-
ples collected at the time of enrollment from June 2016 to March 2020 were included. Of these patients, nine with percutaneous transluminal angioplasty within 3 months before study enrollment were excluded because percutaneous transluminal angioplasty might affect the plasma levels of endostatin via endothelial stimulation. Therefore, 372 patients on HD were enrolled in this study. The study population was divided into three groups based on plasma endostatin levels, as follows: tertile 1, <146 ng/mL; tertile 2, 146–196 ng/mL; and tertile 3, ≥196 ng/mL. All participants were followed up prospectively after baseline characteristics were assessed. Patient follow-up was censored at the time of transfer to peritoneal dialysis, renal transplantation, follow-up loss, or withdrawal of patient consent.

The study protocol was approved by the Institutional Review Board of Kyung Hee University Hospital (No. KHN-MC 2016-04-039) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants involved in this study.

Data collection and outcome measures

General demographic information, prior medical history, cause of renal disease, comorbid conditions, laboratory data, drug use, vascular assessment, and dialysis information were collected from the medical records and interviews. Single-pool Kt/V (spKt/V) (where K, dialyzer clearance; t, time; and V, urea distribution volume) was assessed using the conventional method \[13\], and body mass index (BMI) was calculated from the patient's weight (kg) divided by the square of body height (m\(^2\)) in a standard manner. Information on comorbidities was assessed using the Charlson comorbidity index score \[18\].

The primary study outcome was a composite of incident CV events, including both cardiac and non-cardiac vascular events. Cardiac events included acute coronary artery syndrome, heart failure, ventricular arrhythmia, cardiac arrest, and sudden death. Non-cardiac vascular events were defined as cerebral infarction, cerebral hemorrhage, and peripheral vascular occlusive diseases that required revascularization or surgical intervention. All-cause mortality was recorded and carefully reviewed.

Laboratory analysis

Blood samples for laboratory analysis were collected in a fasting state before HD during a midweek session on the same day the baseline clinical characteristics were collected. After centrifugation at 1,000× g at room temperature for 15 minutes, the samples were stored at −80 °C until analysis. Routine biochemical parameters were measured using standard laboratory methods. Plasma levels of endostatin, osteoprotegerin, and receptor activator of nuclear factor kappa-B ligand (RANKL) were measured by enzyme-linked immunosorbent assay using Magnetic Luminex Screening Assay multiplex kits (R&D Systems, Inc.). Osteoprotegerin and RANKL levels were measured in 275 patients (73.9%), depending on sample availability.

Vascular calcification assessment

To assess the severity of vascular calcification, we used a scoring system for abdominal aortic calcification based on lateral lumbar radiography. Vascular calcification scores were estimated using a previously reported method \[19\] and interpreted by nephrologists who were blinded to the clinical data of the participants. The abdominal aortic walls were divided into four sections corresponding to the lumbar spine from the first (L1) to the fourth (L4) vertebrae, and each aortic wall was scored from 0 to 3 points. The abdominal aortic calcification score represents the composite score of calcification grades of the anterior and posterior abdominal aortic walls, with a maximum score of 24 points. Among all studied patients, 275 (73.9%) underwent radiographic examinations for vascular calcification assessment.

Statistical analysis

Continuous variables are presented as mean ± standard deviation values or median (interquartile range [IQR]) values, and categorical variables are presented as frequencies and percentages. Differences among the three groups were identified using analysis of variance or the Kruskal-Wallis test. The Tukey post-hoc test and Mann-Whitney U test with Bonferroni correction were used to identify intergroup differences. Categorical data were analyzed using the chi-square test or Fisher exact test. Correlations between continuous variables were evaluated using Spearman cor-
relation analysis. A Cox proportional hazards model was constructed to identify the independent variables related to CV events and all-cause mortality. The multivariate models included parameters that were significantly associated with weight in univariate testing and clinically fundamental parameters. The parameters included in the multivariate Cox analysis were age, sex, BMI, Charlson comorbidity index, dialysis duration, hemoglobin, high-sensitivity C-reactive protein, angiotensin receptor blocker or angiotensin-converting enzyme inhibitor use, and spKt/V. Statistical analyses were conducted using the IBM SPSS version 22.0 (IBM Corp.), and p < 0.05 was considered to indicate statistical significance.

Results

Baseline demographic characteristics and laboratory data

The median circulating endostatin level was 171.0 ng/mL (IQR, 117.0–209.8 ng/mL) among all study participants. The median endostatin level was 105.0 ng/mL (IQR, 87.5–117.0 ng/mL) in tertile 1 (n = 124), 171.0 ng/mL (IQR, 160.0–183.0 ng/mL) in tertile 2 (n = 124), and 234.5 ng/mL (IQR, 209.3–259.8 ng/mL) in tertile 3 (n = 124), respectively. The baseline characteristics and laboratory results of the study population across tertiles of endostatin use are summarized in Table 1. Patients in tertile 1 had a shorter dialysis duration than those in tertiles 2 and 3. Laboratory parameters and dialysis characteristics did not differ significantly between the two groups. The plasma levels of the MBD markers did not differ across the endostatin tertiles.

Determinant factors of higher endostatin level

Univariate and multivariate logistic regression analyses of endostatin tertile 3 and the baseline parameters are shown in Table 2. Low-density lipoprotein (LDL) cholesterol levels and predialysis systolic blood pressure were marginally associated with endostatin tertile 3 in the univariate analysis. In the multivariate analysis, LDL cholesterol levels (odds ratio [OR], 1.01; 95% confidence interval [CI], 1.00–1.02; p = 0.04) and predialysis systolic blood pressure (OR, 1.01; 95% CI, 1.00–1.02; p = 0.04) were significant determinants of endostatin tertile 3.

Correlation of endostatin level with circulating mineral bone disease biomarkers and baseline characteristics

Table 3 shows the correlations between endostatin levels and circulating MBD marker levels. The plasma levels of endostatin did not significantly correlate with calcium × phosphorous, intact parathyroid hormone, osteoprotegerin, or RANKL. There was also no significant correlation between vascular calcification score in the abdominal aorta and endostatin level.

Prognostic utility of endostatin level in patients undergoing hemodialysis

During a mean follow-up of 30.5 months, 54 CV events (14.5%) and 44 cardiac events (11.8%) occurred. The cumulative event rate for CV events was significantly higher as endostatin levels increased, with endostatin tertile 3 having the highest cumulative CV event rate (p = 0.006) (Fig. 1A). Endostatin tertile 3 was associated with a greater cumulative rate of cardiac events (p = 0.04) (Fig. 1B).

Table 4 shows the hazard ratios (HRs) of plasma endostatin for the CV events. Univariate Cox regression analysis revealed that endostatin tertile 3 was significantly associated with an increased risk of composite CV events (HR, 3.89; 95% CI, 1.60–9.43; p = 0.003), and this association remained significant after adjustment for multiple variables (HR, 4.37; 95% CI, 1.79–10.67; p = 0.001). Endostatin increments per 1 ng/mL were independently associated with an increased risk of composite CV events (HR, 1.004; 95% CI, 1.001–1.007; p = 0.02). To further investigate the risk of composite CV events, the HRs for cardiac and non-cardiac vascular events were analyzed. Patients in tertile 3 had a significant risk of cardiac events even after adjusting for multiple variables (HR, 3.88; 95% CI, 1.44–10.44; p = 0.007), and endostatin increment per 1 ng/mL was also associated with a higher risk of CV events (HR, 1.005; 95% CI, 1.001–1.008; p = 0.02). However, endostatin levels were not associated with a significant risk of non-cardiac vascular events.

Discussion

In this study, we investigated the association between circulating endostatin levels and CV and MBD parameters in patients with ESRD undergoing HD using a prospective
Table 1. Baseline demographics and laboratory data of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tertile 1a</th>
<th>Tertile 2b</th>
<th>Tertile 3c</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>61.4 ± 12.9</td>
<td>61.3 ± 12.5</td>
<td>61.0 ± 12.7</td>
<td>0.73</td>
</tr>
<tr>
<td>Male sex</td>
<td>78 (62.9)</td>
<td>81 (65.3)</td>
<td>83 (66.9)</td>
<td>0.80</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2 ± 4.5</td>
<td>23.4 ± 4.2</td>
<td>23.4 ± 3.9</td>
<td>0.89</td>
</tr>
<tr>
<td>Dialysis duration (yr)</td>
<td>2.3 ± 3.9†</td>
<td>5.2 ± 6.6*</td>
<td>4.0 ± 5.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CCI score</td>
<td>4.1 ± 1.4</td>
<td>4.1 ± 1.6</td>
<td>4.1 ± 1.6</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Causes of renal disease</td>
<td></td>
<td></td>
<td></td>
<td>0.87</td>
</tr>
<tr>
<td>Diabetes</td>
<td>63 (50.8)</td>
<td>58 (46.8)</td>
<td>54 (43.5)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>19 (15.3)</td>
<td>28 (22.6)</td>
<td>25 (20.2)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>15 (12.1)</td>
<td>13 (10.5)</td>
<td>17 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Polycystic kidney</td>
<td>6 (4.8)</td>
<td>8 (6.5)</td>
<td>7 (5.6)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>21 (16.9)</td>
<td>17 (13.7)</td>
<td>21 (16.9)</td>
<td></td>
</tr>
<tr>
<td>CV history</td>
<td>51 (41.1)</td>
<td>55 (44.4)</td>
<td>49 (39.5)</td>
<td>0.73</td>
</tr>
<tr>
<td>Malignancy history</td>
<td>13 (10.5)</td>
<td>12 (9.7)</td>
<td>6 (4.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6 ± 1.3</td>
<td>10.4 ± 1.2</td>
<td>10.4 ± 1.1</td>
<td>0.38</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.78 ± 0.32</td>
<td>3.82 ± 0.32</td>
<td>3.82 ± 0.31</td>
<td>0.44</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>76.4 ± 27.1</td>
<td>73.3 ± 23.9</td>
<td>79.6 ± 27.3</td>
<td>0.17</td>
</tr>
<tr>
<td>hsCRP (mg/dL)</td>
<td>3.9 ± 7.8</td>
<td>4.9 ± 8.5</td>
<td>3.7 ± 7.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>8.4 ± 0.8</td>
<td>8.6 ± 0.8</td>
<td>8.5 ± 0.8</td>
<td>0.18</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.9 ± 1.3</td>
<td>5.0 ± 1.2</td>
<td>5.0 ± 1.4</td>
<td>0.90</td>
</tr>
<tr>
<td>Ca × P (mg²/dL²)</td>
<td>41.3 ± 12.2</td>
<td>42.5 ± 11.6</td>
<td>42.5 ± 11.9</td>
<td>0.64</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>106.0 ± 88.5</td>
<td>90.0 ± 39.0</td>
<td>91.5 ± 52.9</td>
<td>0.09</td>
</tr>
<tr>
<td>i-PTH (pg/mL)</td>
<td>282.4 ± 203.1</td>
<td>290.3 ± 240.5</td>
<td>311.5 ± 227.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Osteoprotegerin (ng/mL)</td>
<td>2.76 (18.56–36.23)</td>
<td>2.99 (2.29–4.08)</td>
<td>2.96 (1.88–3.71)</td>
<td>0.17</td>
</tr>
<tr>
<td>RANKL (ng/mL)</td>
<td>8.11 (1.32–22.26)</td>
<td>8.11 (1.50–24.14)</td>
<td>14.41 (1.50–28.93)</td>
<td>0.25</td>
</tr>
<tr>
<td>Predialysis SBP (mmHg)</td>
<td>141.4 ± 20.1</td>
<td>141.4 ± 19.0</td>
<td>145.6 ± 18.8</td>
<td>0.14</td>
</tr>
<tr>
<td>spKt/V</td>
<td>1.59 ± 0.28</td>
<td>1.58 ± 0.28</td>
<td>1.61 ± 0.31</td>
<td>0.70</td>
</tr>
<tr>
<td>Ultrafiltration (L)</td>
<td>2.05 ± 1.06</td>
<td>2.29 ± 0.99</td>
<td>2.28 ± 0.94</td>
<td>0.11</td>
</tr>
<tr>
<td>Catheter as vascular access</td>
<td>7 (5.6)</td>
<td>4 (3.2)</td>
<td>6 (4.8)</td>
<td>0.74</td>
</tr>
<tr>
<td>Hemodiafiltration</td>
<td>40 (32.3)</td>
<td>32 (25.8)</td>
<td>35 (28.2)</td>
<td>0.53</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI or ARB</td>
<td>67 (54.0)</td>
<td>73 (58.9)</td>
<td>88 (71.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Statin</td>
<td>67 (54.0)</td>
<td>62 (50.0)</td>
<td>54 (43.5)</td>
<td>0.25</td>
</tr>
<tr>
<td>Anti-platelet</td>
<td>91 (73.4)</td>
<td>97 (78.2)</td>
<td>88 (71.0)</td>
<td>0.41</td>
</tr>
<tr>
<td>Vascular calcification score in the abdominal aorta</td>
<td>7.0 ± 6.2</td>
<td>7.2 ± 6.6</td>
<td>7.2 ± 7.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Follow-up (mo)</td>
<td>25.6 ± 13.9†</td>
<td>33.2 ± 13.7*</td>
<td>32.6 ± 14.3*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as number only, mean ± standard deviation, number (%), or median (IQR).

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; Ca, calcium; CCI, Charlson comorbidity index; CV, cardiovascular; hsCRP, high-sensitivity C-reactive protein; IQR, interquartile range; i-PTH, intact parathyroid hormone; LDL, low-density lipoprotein; P, phosphorus; RANKL, receptor activator of nuclear factor kappa-B ligand; SBP, systolic blood pressure; spKt/V, single-pool Kt/V.

a–c Endostatin level (ng/mL): a<146 (median, 105.0; IQR, 87.5–117.0), b146–196 (median, 171.0; IQR, 160.0–183.0), and c≥196 (median, 234.5; IQR, 209.3–259.8).

†p < 0.05 vs. tertile 1; *p < 0.05 vs. tertile 2.
### Table 2. Logistic regression analysis of the relationship between baseline parameters and endostatin tertile 3 status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.99 (0.98–1.01)</td>
<td>0.43</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.13 (0.72–1.79)</td>
<td>0.59</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.01 (0.96–1.07)</td>
<td>0.65</td>
</tr>
<tr>
<td>Dialysis duration</td>
<td>1.01 (0.97–1.05)</td>
<td>0.64</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.91 (0.59–1.40)</td>
<td>0.66</td>
</tr>
<tr>
<td>CV history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.92 (0.77–1.09)</td>
<td>0.33</td>
</tr>
<tr>
<td>Albumin</td>
<td>1.27 (0.64–2.55)</td>
<td>0.497</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.01 (1.00–1.02)</td>
<td>0.11</td>
</tr>
<tr>
<td>hsCRP</td>
<td>0.99 (0.96–1.02)</td>
<td>0.45</td>
</tr>
<tr>
<td>Predialysis SBP</td>
<td>1.01 (1.00–1.02)</td>
<td>0.05</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>1.12 (0.90–1.39)</td>
<td>0.31</td>
</tr>
<tr>
<td>Catheter</td>
<td>1.10 (0.40–3.04)</td>
<td>0.86</td>
</tr>
<tr>
<td>Hemodiafiltration</td>
<td>0.96 (0.60–1.55)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

CI, confidence interval; CV, cardiovascular; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; OR, odds ratio; SBP, systolic blood pressure.

### Table 3. Correlation between endostatin level and circulating MBD biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Correlation coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca × P (mg²/dL²)</td>
<td>0.025</td>
<td>0.63</td>
</tr>
<tr>
<td>i-PTH (pg/mL)</td>
<td>0.025</td>
<td>0.63</td>
</tr>
<tr>
<td>Osteoprotegerin (ng/mL)</td>
<td>0.002</td>
<td>0.97</td>
</tr>
<tr>
<td>RANKL (ng/mL)</td>
<td>0.078</td>
<td>0.20</td>
</tr>
<tr>
<td>Vascular calcification score in abdominal aorta</td>
<td>-0.006</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Ca, calcium; i-PTH, intact parathyroid hormone; MBD, mineral bone disease; P, phosphorus; RANKL, receptor activator of nuclear factor kappa-8 ligand.

Observational cohort database. Endostatin levels were significantly correlated with LDL cholesterol levels and predialysis systolic blood pressure, which are conventional risk factors for atherosclerotic CV events. Higher circulating endostatin levels were significantly associated with an increased risk of incident CV composites and cardiac events, even after adjusting for multiple variables. However, we did not observe a correlation between endostatin levels and MBD markers or vascular calcification scores in this study.

Previous studies have reported elevated plasma endostatin levels in various disease conditions and have demonstrated that plasma endostatin levels are significantly correlated with disease severity and clinical outcomes [20]. The median circulating endostatin level in our study was 171.0 ng/mL, which was relatively increased considering that the level in healthy individuals ranges from 24.6 to 136.1 ng/mL [21,22]. Chen et al. [23] documented significantly increased plasma endostatin levels in patients with non-dialysis-dependent CKD compared to controls without CKD. They also observed a concentration-dependent relationship between the severity of CKD and plasma endostatin levels. In addition, it seems that the endostatin levels in this study were higher in our study group than in the normal controls and patients with non-dialysis-dependent CKD, as measured in previous studies [23,24]. These findings suggest that endostatin levels increase as renal function decreases. Further studies are required to investigate whether increased endostatin levels with declining renal function are derived from impaired renal excretion or pathological conditions related to lower renal function.

Studies have shown that higher serum endostatin levels in patients with coronary artery disease are associated with reduced angiogenesis and poorly developed collateral vasculature [25]. Circulating endostatin levels predict the development of CV diseases such as recurrent ischemic stroke [26] and incident myocardial infarction [27]. High plasma endostatin levels in patients with stable coronary heart disease reflect CV and total mortality [28]. Two independent community-based cohorts showed a significant
Figure 1. Cumulative cardiovascular (A) and cardiac (B) event rates according to the endostatin levels.

Table 4. HR of plasma endostatin level for CV event

<table>
<thead>
<tr>
<th>Endostatin level</th>
<th>No. of events (%)</th>
<th>Composite of CV event</th>
<th>Cardiac event</th>
<th>Non-cardiac vascular event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endostatin tertile 1</td>
<td>6 (4.8)</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endostatin tertile 2</td>
<td>21 (16.9)</td>
<td>2.81 (1.13–6.98)</td>
<td>3.02 (1.13–8.11)</td>
<td>1.70 (0.31–9.30)</td>
</tr>
<tr>
<td>Endostatin tertile 3</td>
<td>27 (21.8)</td>
<td>3.89 (1.60–9.43)</td>
<td>3.34 (1.25–8.92)</td>
<td>3.55 (0.75–16.77)</td>
</tr>
<tr>
<td>Endostatin per 1 ng/mL</td>
<td>1.005 (1.001–1.008)</td>
<td>1.005 (1.001–1.008)</td>
<td>1.005 (1.001–1.008)</td>
<td>1.005 (0.999–1.011)</td>
</tr>
</tbody>
</table>

All analyses were adjusted for the following covariates: age, sex, body mass index, Charlson comorbidity index, dialysis duration, hemoglobin, high-sensitivity C-reactive protein, angiotensin receptor blocker or angiotensin-converting enzyme inhibitor use, and single-pool Kt/V.

CI, confidence interval; CV, cardiovascular; HR, hazard ratio.

association between CV mortality and plasma endostatin levels [29]. Higher circulating endostatin levels may be involved in inflammatory stress and active extracellular remodeling [30]. The inflammatory process in hypoxia or ischemia, which frequently occurs in patients undergoing HD, stimulates metalloproteinase, elastase, and cathepsins, and they can modulate the extracellular matrix and release endostatin [31]. In addition, human endothelial cells incubated with high endostatin levels can show more apoptotic changes [32]. Endostatin-induced endothelial apoptosis may correlate with myocardial rarefaction and CV dysfunction [33]. Circulating endostatin levels are also associated with a higher risk of CV events in patients with CKD [34]. In line with these findings, we demonstrated the significant predictive value of plasma endostatin levels for CV events in patients with ESRD on HD. Furthermore, our
results showed that the circulating endostatin level was an independent predictive factor for the occurrence of cardiac events. These findings suggest that the association between higher levels of circulating endostatin and a greater risk of atherosclerotic events remains significant in patients receiving HD treatment and that predictive values of endostatin could be expanded to several categories of diseases.

Endostatin has a complex biology. Animal studies have identified a protective role of endostatin in the development of CV disease. Endostatin neutralization by the injection of anti-endostatin antibody in myocardial infarction–induced rats deteriorated left ventricular remodeling [35], and endostatin inhibition attenuated inflammation, limited oxidative stress, and reduced plaque growth and atherosclerosis [36,37]. In addition, endostatin mediates a major blocking effect on LDL cholesterol retention in atherosclerosis-prone mice, and the infusion of endostatin lowers blood pressure and vasorelaxation [38,39]. Our results also showed that LDL cholesterol and systolic blood pressure are independent determinants of higher endostatin levels and that endostatin tertile 3 was associated with an increased risk of CV and cardiac events in patients on HD. Considering these findings, we suggest that endostatin has the therapeutic potential to prevent CV complications in patients on HD and that clinical studies are warranted to investigate the role of endostatin treatment.

In this study, we expected that endostatin levels would show a positive correlation with circulating MBD markers and vascular calcification score because increased endostatin levels are reported to be associated with coronary or aortic valve calcification [9]. However, we did not observe any correlation between endostatin levels and MBD markers or vascular calcification scores. We presumed that medial vascular calcification induced no relationship between endostatin levels and vascular calcification. MBD is the main contributor to calcific deposition on the medial arterial layer, and patients with ESRD face a considerable burden brought on by the medial type of vascular calcification [2]. While these patients may have had an intimal type of vascular calcification, the additional occurrence of medial vascular calcification from MBD may have influenced the vascular calcification scores. We speculated that circulating endostatin levels could be more closely associated with atherosclerotic intimal vascular calcification than with medial vascular calcification.

This study had some limitations. We could not perform separate analyses for specific CV events due to the limited number of events. As this was a retrospective study, there may have been unintended bias. In addition, circulating osteoprotegerin levels, RANKL levels, and vascular calcification scores were not obtained for all enrolled patients. When the study population was divided by the median level of endostatin, the results of the univariate and multivariate analyses for the composite of CV events were as follows: HR, 1.31; 95% CI, 0.76–2.26; p = 0.34 and HR, 1.21; 95% CI, 0.69–2.11; p = 0.50, respectively. Considering these findings, it is difficult to suggest the optimal cutoff value of circulating endostatin for CV event prediction based on our study. Finally, the intimal and medial types of vascular calcification were not differentiated because a simple radiological examination is limited in its capacity to assess vascular calcification type.

In conclusion, circulating endostatin levels were significantly correlated with LDL cholesterol levels and predialysis systolic blood pressure but did not correlate with MBD markers or vascular calcification. Higher circulating endostatin levels were associated with a greater risk of composite CV and cardiac events in patients with ESRD on HD. Our findings indicate that endostatin is a biomarker that can help predict CV events in patients with ESRD undergoing HD.

Conflicts of interest
All authors have no conflicts of interest to declare.

Data sharing statement
The data presented in this study are available upon reasonable request from the corresponding author.

Authors’ contributions
Conceptualization: JSK, MK, YGK, HSH
Data curation: KHJ, JYM, GJK, SYL
Formal analysis: JSK, MK, SHL, DYL, YGK
Methodology: JSK, MK, KHJ, JYM, DYL, SYL, YGK, HSH
Supervision: KHJ, JYM, SHL, GJK, DYL, SYL
Visualization: JSK, MK, DYL, SYL, YGK, HSH
Writing–original draft: JSK, MK
Writing–review & editing: KHJ, SHL, GJK, YGK, HSH
All authors read and approved the final manuscript.

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References


Prediction of diabetes mellitus after kidney transplantation using patient-specific induced pluripotent stem cells

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**Background:** Multiple risk factors are involved in new-onset diabetes mellitus (DM) after organ transplantation; however, their ability to predict clinical prognosis remains unclear. Therefore, we investigated whether patient-specific induced pluripotent stem cells (iPSCs) could help predict DM development before performing kidney transplantation (KT).

**Methods:** We first performed whole transcriptome and functional enrichment analyses of KT patient-derived iPSCs. Our results revealed that insulin resistance, type 2 DM, and transforming growth factor beta signaling pathways are associated between the groups of DM and non-DM. We next determined whether the genetic background was associated with development of iPSCs into pancreatic progenitor (PP) cells.

**Results:** The levels of differentiation-related key markers of PP cells were significantly lower in the DM group than in the non-DM group. Moreover, the results of tacrolimus toxicity screening showed a significant decrease in the number of PP cells of the DM group compared with the non-DM group, suggesting that these cells are more susceptible to tacrolimus toxicity.

**Conclusion:** Taken together, these results indicate that PP cells of the DM group showed low developmental potency accompanied by a significantly different genetic background compared with the non-DM group. Thus, genetic analysis can be used to predict the risk of DM before KT.

**Keywords:** Induced pluripotent stem cells, Kidney transplantation, Diabetes mellitus, Insulin secreting cells, Tacrolimus

**Introduction**

The incidence rate of new-onset diabetes mellitus (DM) after kidney transplantation (KT) at 12 months posttransplant is 20% to 50%. DM after transplantation is associated with increased risks of graft rejection, infection, cardiovascular disease, and death [1–3]. Multiple risk factors have been implicated in DM development. Non-modifiable risk factors for new-onset DM include older age; African American, Hispanic, or South Asian ethnicity; genetic background; positive family history of DM; polycystic kidney disease; and previously diagnosed glucose intolerance.
Modifiable risk factors for new-onset DM after KT include obesity; metabolic syndrome; hepatitis C virus or cytomegalovirus infection; and therapy with corticosteroids, calcineurin-inhibitor drugs (especially tacrolimus [Tac]), or sirolimus [4].

Induced pluripotent stem cell (iPSC) technology has been developed to differentiate iPSCs into almost any organ-specific cell type. This technology may enable the generation of disease-relevant tissues from patients in scalable quantities. The use of patient-derived iPSCs has helped in the research of pathophysiological mechanisms of various diseases. The iPSC-derived organs and organoids are also currently being evaluated in regenerative therapy, which is proceeding toward clinical trials, and disease modeling, which facilitates drug-screening efforts for discovering novel therapeutics [5,6].

Therefore, we designed this study to investigate the feasibility of a novel DM prediction model involving patient iPSC cells. We reprogrammed patient-specific iPSCs from peripheral blood mononuclear cells (PBMCs) before performing KT. At approximately 1 year after KT, we compared the genetic differences between the DM and non-DM groups by RNA-sequencing analysis to reveal genetic links with insufficient pancreatic beta cell function or maturation.

Pancreatic progenitor (PP) cells, which are multipotent cells with the potential to develop into endocrine, exocrine, or epithelial cells, provide a powerful model system for examining the molecular characteristics of differentiating fetal-like pancreatic cells and for genetic analysis of pancreatic disease [7–9]. Therefore, we next compared the differentiation potential of PP cells between the DM and non-DM groups by evaluating the morphology and differentiation of marker expression.

To determine whether the DM group after KT is likely to be susceptible to immunosuppressive agents, we also tested cell viability and insulin expression of PP cells from the DM and non-DM groups during Tac-induced toxicity. Our results showed that the PP cells of the DM group showed low developmental potency accompanied by a significantly different genetic background compared with the non-DM group. We expect that the results of our study will provide a rationale for prediction of the risk of DM after KT.

**Methods**

**Study population**

The Institutional Review Board of The Catholic University of Korea, Seoul St. Mary’s Hospital approved this study (No. KC16TISI0774). Formal informed consent was obtained from the patients. We recruited pre-KT patients (n = 20) who had never been treated with anti-DM medication and who had provided PBMCs for experiments. Among the 20 patients, five were diagnosed with DM at 1 year after KT; we selected matched patients with DM (n = 4) and non-DM individuals (n = 4) based on the clinical index (Supplementary Table 1, 2; available online).

**Induced pluripotent stem cell differentiation**

The iPSCs from four patients with DM and four non-DM individuals were generated using PBMCs, as previously described [10]. Briefly, PBMCs were cultured for 4 days at 37 °C in an incubator with 5% CO₂ in StemSpan medium (09650; STEMCELL Technologies), which includes StemSpan CC100 (02690; STEMCELL Technologies), to expand CD34-positive cells. The expanded PBMCs were transfectedusing the CytoTune-iPS Sendai Reprogramming Kit (A16517; Life Technologies), which includes the Yamanaka factors (Oct4, Sox2, KLF4, and c-Myc). PBMCs were induced to form iPSCs via centrifugation; the resultant attached cells were expanded and purified by colony picking.

**Pancreatic progenitor cell differentiation**

Human iPSCs were subcultured in dishes coated with Matrigel (354277; Corning Life Sciences) at 37 °C in an incubator with 5% CO₂. Fresh mTeSR1 medium (05850; STEMCELL Technologies) was used as the culture medium and was replaced daily. The iPSCs were split using trypsin-ethylenediaminetetraacetic acid (TE) (15400054; Life Technologies) at 70% confluence, and 10 μM of a rho-associated kinase inhibitor (1254; TOCRIS Bioscience) was added to the newly passaged cells. The STEMdiff PP kit (05120; STEMCELL Technologies) provided the culture medium for differentiation into PP cells.
Cell Counting Kit-8 assay

The iPSC-derived PP cells were differentiated in 96-well microplates for the Cell Counting Kit-8 (CCK-8) assay. After differentiation, the cells were subjected to various Tac treatments for specified durations. CCK-8 solution (CK04-01; Dojindo Molecular Technologies) was added to each well for 2 hours. Absorbance was measured at 450 nm using a VersaMax ELISA Reader (Molecular Devices).

Quantitative real-time-polymerase chain reaction

RNA was extracted from iPSCs or PP cells using RNA-Bee (CD-105B; Tel-Test), as per the manufacturer’s instructions. First-strand complementary DNA was synthesized and subjected to quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green Master Mix (DYRT1200; Dyne Bio Inc.) in a LightCycler 480 system (Roche). Target gene expression was normalized to that of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the change-in-threshold method. Primer sequences are listed in Supplementary Table 3 (available online).

Flow cytometry

The iPSCs or iPSC-derived PP cells were dissociated using TE (15400054). The cells were washed twice with FACS buffer (phosphate-buffered saline [PBS] containing 1% bovine serum albumin and 10-mM sodium azide), permeabilized for 30 minutes using flow cytometry fixation and permeabilization solution (554714; BD Biosciences), washed with wash buffer, stained with anti-OCT3/4 (60093AD.1; STEMCELL Technologies) and anti-insulin (565689; BD Biosciences) antibodies for 1 hour each, and then washed with FACS buffer. Samples were analyzed using a BD LSRFortessa cell analyzer (BD Biosciences). Data were analyzed using the FlowJo V10 Single Cell Analysis Software (TreeStar Inc.).

Suspension culture of pancreatic progenitor cells

For further maturation of PP cells, suspension culture was performed as previously described [11]. PP cells were treated with 5 mg/mL dispase (07913; STEMCELL Technologies) for 5 minutes, followed by gentle pipetting to obtain cell clumps (<100 µm). The cell clusters were transferred into a polystyrene 125 mL Spinner Flask (3152; Corning Life Sciences) and spun at 80 to 100 rpm overnight in suspension with DMEM-HG (10-017-CV; Corning Life Science) supplemented with 1-µmol/L ALK5 inhibitor II (ALX-270-445-M005; Enzo Life Sciences), 100-ng/mL Noggin (6057-NG-100; R&D Systems), and 1% B27 (17504077; Life Technologies).

Immunofluorescence staining

Cell clusters were obtained in 1.5 mL tubes after suspension culture for insulin staining. The cell clusters were then incubated with 4% paraformaldehyde for 15 minutes at 4 °C and washed three times in PBS at room temperature (RT). Cells were then incubated with 0.1% Triton X-100 for 10 minutes and 10% normal donkey serum for 1 hour at RT. Samples were then incubated with primary antibodies, anti-insulin (18-0067; Invitrogen), anti-OCT3/4 (5279; Santa Cruz Biotechnology), anti-SOX2 (365823; Santa Cruz Biotechnology), and anti-SSEA4 (MAB4304; Millipore Sigma) antibodies, at 4 °C overnight. The following day, cells were incubated with a secondary Cyanine3 (Jackson ImmunoResearch)-conjugated antibody for 2 hours at RT. Cells were then stained with 4’,6-diamidine-2-phenylindole (DAPI; Vector Laboratories) for nucleic acid staining. Images were obtained using a Zeiss LSM700 confocal microscope (Carl Zeiss MicroImaging GmbH).

Electron microscopy

PP cells were fixed in 2.5% glutaraldehyde, 0.1 M phosphate buffer, and 1% O₃O₂ and then embedded in Epon 812. Ultrathin sections were cut, stained with uranyl acetate/lead citrate, and photographed under a JEM-1200EX transmission electron microscope (JEOL Ltd.). The sections were randomly scanned at 20 spots per sample at 5,000× magnification.

Library preparation and sequencing

For control and test RNAs, library construction was performed using the QuantSeq 3' mRNA-Seq Library Prep Kit (Lexogen, Inc.), according to the manufacturer’s instructions. In brief, total RNA samples (500 ng each) were pre-
pared; an oligo-dT primer containing an Illumina-compatible sequence at its 5’ end was hybridized to the RNA and reverse transcribed. After RNA template degradation, second-strand synthesis was initiated by a random primer containing an Illumina-compatible linker sequence at its 5’ end. The double-stranded library was purified using magnetic beads to remove all reaction components. The library was amplified to add the complete adapter sequences required for cluster generation. The final library was purified from the PCR components. High-throughput sequencing was performed as single-end 75-bp sequencing using a NextSeq 500 system (Illumina, Inc.).

Data analysis

QuantSeq 3’ mRNA-Seq reads were aligned using Bowtie2 [12]. Bowtie2 indices were either generated from the genome assembly sequence or representative transcript sequences for alignment to the genome and transcriptome. The alignment file was used for assembling transcripts, estimating their abundance, and detecting differential gene expression. Differentially expressed genes (DEGs) were identified on the basis of counts from unique and multiple alignments using coverage in BEDtools [13]. The read count data were processed on the basis of the TMM + CPM normalization method with EdgeR within R (R Development Core Team, 2020) using Bioconductor [14]. Gene classification was based on searches performed in the DAVID (https://david.ncifcrf.gov/home.jsp) and Medline databases (https://www.ncbi.nlm.nih.gov/).

Statistical analyses

Data are expressed as mean ± standard error of at least three independent experiments. Multiple comparisons between groups were performed by one-way analysis of variance with the Bonferroni post hoc test using the Prism software (version 7.03 for Windows; GraphPad Software). Statistical significance was set at p < 0.05.

Results

Generation of induced pluripotent stem cells from peripheral blood mononuclear cells of pre-kidney transplantation patients

PBMCs were induced to form iPSCs using Sendai viruses expressing Yamanaka factors (Oct4, Sox2, KLF4, and c-Myc). The reprogramming method was based on a previously described protocol involving serial centrifugation [10]. Colonies were generated from somatic cells after approximately 18 days. PCR analysis revealed that iPSCs expressed GAPDH, OCT3/4, SOX2, NANOG, LIN28, DPPA5, and TDGF1 messenger RNAs (mRNAs) (Fig. 1A). Flow cytometry revealed that approximately 90% of the iPSCs were positive for the pluripotency marker OCT3/4 (Fig. 1B). In addition, we confirmed the expression of the pluripotency markers OCT3/4, SOX2, SSEA4, KLF4, TRA-1-61, and TRA-1-81 using immunofluorescence (Fig. 1C; Supplementary Fig. 1A, Supplementary Methods, available online). To confirm that the iPSCs generated were genotypically normal, we analyzed their karyotypes using the Giemsa-trypsin-Giemsa banding method. The iPSCs showed a normal karyotype of 44 + XX or 44 + XY, except in the case of DM 3 (trisomy 20) (Supplementary Fig. 1B, Supplementary Methods, available online).

Gene and functional enrichment analyses in patient-specific induced pluripotent stem cells of the diabetes mellitus group

To investigate the differences in the gene expression profiles of patient-specific iPSCs between the DM and non-DM groups, we performed transcriptomic analysis using RNA-seq. RNA-seq analysis was used to identify DEGs on the basis of the DM:non-DM ratio. A total of 242 DEGs with a p-value of <0.05 were identified, of which 187 genes showed two-fold upregulation and 55 genes showed two-fold downregulation (Fig. 2A). A volcano plot of the RNA-seq results illustrating the DEG findings with respect to the DM:non-DM ratio is shown in Fig. 2B. On plotting the hierarchical clustering heat map of all DEGs, we found that most DEGs showed consistently higher or lower expression in individuals with DM (Fig. 2C).

Kyoto Encyclopedia of Genes and Genomes (KEGG) en-
Figure 1. The iPSC generation from pre-kidney transplantation patients without diabetes. (A) Quantitative real-time polymerase chain reaction data for pluripotency gene expression in iPSCs. (B) Flow cytometry data for iPSCs, showing an OCT4-positive cell population. (C) Immunocytochemistry images showing that pluripotency markers (OCT4, Sox2, and SSEA4) were expressed in iPSCs. Data are expressed as mean ± standard error. Scale bar, 100 μm.

DM, diabetes mellitus; iPSC, induced pluripotent stem cell; mRNA, messenger RNA.
Figure 2. Differentially expressed gene (DEG) analysis with the DM:non-DM ratio. (A) Two-fold upregulated genes (187 genes) and two-fold downregulated genes (55 genes). (B) Volcano plot showing the DEGs. The x-axis represents the log2 fold change conversion of the values, and the y-axis represents the significance value after -log10 conversion. Red dots indicate 187 upregulated DEGs, blue dots indicate 55 downregulated DEGs, and the gray area represents no DEGs. (C) Heat map showing the differential expression pattern of the DEGs. The color scale shows the gene expression values (log2fc). Kyoto Encyclopedia of Genes and Genomes pathway analyses of DEGs and top 12 pathway by p-value. (D) Bar graph represents up- and downregulated DEGs. Y-axis represents pathway name, and x-axis represents the number (count) of genes or -log10 (p-value). (E) Size and color of each bubble represent the number of DEGs enriched in the pathway and -log10 (p-value), respectively. Y-axis represents pathway name, and x-axis represents fold enrichment factor. cGMP-PKG, cGMP-dependent protein kinase G; DM, diabetes mellitus; TGF-β, transforming growth factor beta.
Figure 3. Statistical comparison of KEGG pathways and validation of RNA-sequencing data by qRT-PCR. RNA-sequencing– and qRT-PCR–based comparisons of the expression of select target differentially expressed genes. DM, diabetes mellitus; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, quantitative real-time polymerase chain reaction.

Figure 4. Differentiation of induced pluripotent stem cells derived from patients with DM and non-DM individuals into pancreatic progenitor cells. (A, B) Overview of the differentiation protocol for 14 days and bright field microscopy image of the cell morphological features at end stage 4 for the groups. Arrows in B indicate pancreatic progenitor (PP) cell formation. Data are expressed as mean ± standard error. Scale bar, 250 μm in A and 200 μm in B. DM, diabetes mellitus.
Figure 5. The mRNA expression levels of genes related to pancreatic beta cell function or differentiation using real-time PCR analysis. The induced pluripotent stem cell-derived pancreatic progenitor (PP) cells from patients with diabetes mellitus (DM) and non-DM individuals. (A) FOXA2, (B) SOX17, (C) GATA4, (D) HNF1B, (E) PDX-1, (F) NKX6.1, (G) SOX9, and (H) NGN3. Data are expressed as mean ± standard error.

Enrichment analysis was performed to predict the potential functions of the DEGs. The top 12 pathways are listed in Fig. 2D and E, and the 29 annotated transcripts pertaining to these pathway terms are indicated in Supplementary Table 4 (available online). Using qRT-PCR, we verified the validity of the annotated transcripts (Fig. 3).

Expression of differentiation markers for pancreatic progenitor cells

We performed qRT-PCR for differentiation markers (FOXA2, SOX17, GATA4, HNF1B, PDX-1, NKX6.1, SOX9, and NGN3) in PP cells in the DM and non-DM groups (Fig. 5). The mRNA expression of these genes in the DM group was significantly lower than that in the non-DM group (Fig. 5) (FOXA2, 17.9 ± 2.2 vs. 54.9 ± 6.1; SOX17, 3.1 ± 0.7 vs. 9.5 ± 2.5; GATA4, 19.0 ± 13.3 vs. 125.4 ± 19.0; HNF1B, 27.8 ± 7.0 vs. 120.0 ± 26.2; PDX-1, 3.6 ± 0.4 vs. 8.3 ± 0.5; NKX6.1, 5.4 ± 0.5 vs. 123.3 ± 63.4; SOX9, 2.3 ± 0.4 vs. 10.8 ± 4.7; and NGN3, 1.8 ± 0.2 vs. 5.5 ± 1.1; p < 0.05 vs. non-DM group in all genes).

Differentiation of induced pluripotent stem cells into pancreatic progenitor cells

To confirm the differentiation of iPSCs into functional endocrine cells, we used a standardized simple protocol to confirm the expression of key markers, including PDX-1, NKX6.1, and SOX9, because insulin-secreting cells from iPSCs are difficult to cultivate in vitro. The protocol involves four stages over 14 days of PP cell formation (Fig. 4A): definitive endoderm (end stage 1), primitive gut tube (end stage 2), posterior foregut endoderm (end stage 3), and PP cells (end stage 4). Representative images of cell morphological features (cell aggregation in high-density regions) at the end of each differentiation stage are shown in Fig. 4B.
Figure 6. The expression of insulin protein and mRNA in iPSC-derived PP cells from patients with DM and non-DM individuals using flow cytometric and real-time PCR analysis. Flow cytometry plots (A) and quantitative graphs (B) for insulin. (C) Insulin mRNA levels in induced pluripotent stem cell-derived PP cells from patients with DM and non-DM individuals. Data are expressed as mean ± standard error.

DM, diabetes mellitus; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; iPSC, induced pluripotent stem cell; mRNA, messenger RNA; PCR, polymerase chain reaction; PP, pancreatic progenitor cell.

Effect of tacrolimus on insulin expression in patient induced pluripotent stem cell-derived pancreatic progenitor cells

Next, we confirmed the insulin levels in the PP cells from each group. Insulin protein and mRNA expression levels were detected via flow cytometry and qRT-PCR, respectively, in the differentiated PP cells. The results showed significantly lower insulin expression in the DM group than in the non-DM group (flow cytometry: 43 ± 7 vs. 66 ± 1, p < 0.05 vs. non-DM group; qRT-PCR: 1.6 ± 0.4 vs. 3.7 ± 0.5, p < 0.05 vs. non-DM group) (Fig. 6). Suspension cultures were performed for PP cells from each group for further maturation. Immunofluorescence results showed that insulin immunoreactivity was lower in the DM group than in the non-DM group (Fig. 7A, B). Electron microscopy (EM) revealed that insulin granules were present in PP cells in the non-DM group but not in those in the DM group (Fig. 7C, D).

Effect of tacrolimus on cell viability in patient induced pluripotent stem cell-derived pancreatic progenitor cells

We examined Tac-induced toxicity in PP cells. We differentiated the patient-specific iPSCs (non-DM individuals, n = 4; DM patients, n = 4) in a 96-well plate using the standardized protocol during the four stages over the 14-day course of PP cell formation. At the end of the differentiation stage, Tac was administered for 24 hours at serial doses of 0, 30,
Figure 7. Representative confocal microscopy images for insulin and transmission electron micrographs in iPSC-derived PP cells from patients with DM and non-DM individuals. (A, B) PP cells were cultured under floating conditions for 1 day and collected for further immunocytochemistry analysis. (C, D) Electron microscopy of iPSC-derived PP cells from patients with DM and non-DM patients. Red arrowheads in C indicate insulin granules. Scale bar in A and B, 50 μm.

DM, diabetes mellitus; iPSC, induced pluripotent stem cell; M, mitochondria. PP, pancreatic progenitor.

40, 50, and 60 μg/mL, and toxicity was confirmed via a cell viability assay using CCK-8 (Fig. 8A). We calculated the area under the curve, indicating the individual cell viability rates at various Tac levels and exposure times (Fig. 8B).

The average cell viability results for each group are shown in Fig. 8C. In PP cells obtained from the DM group during Tac treatment (40- and 50-μg/mL Tac), cell viability was significantly lower than that in the non-DM group (40 μg/mL Tac: 215 ± 5 vs. 271 ± 7, p < 0.05 vs. non-DM group; 50 μg/mL Tac: 121 ± 3 vs. 178 ± 7, p < 0.05 vs. non-DM group). The insulin mRNA levels in the PP cells of the DM group were also markedly lower than those in the non-DM group when 50 μg/mL Tac was used (14 ± 3 vs. 50 ± 4, p < 0.05 vs. non-DM group) (Fig. 8D).

**Discussion**

Our results showed that KT patient-derived iPSCs can be used to predict DM before performing KT. Whole transcriptome and functional enrichment analyses of KT patient-derived iPSCs showed that insulin resistance, type 2 DM, and transforming growth factor beta (TGF-β) signaling pathways are significantly associated between the group of DM and non-DM. The efficiency of differentiation of PP cells from iPSCs was lower in patients with DM than...
Figure 8. Cell viability and insulin levels of iPSC-derived PP cells from patients with DM and non-DM individuals during Tac treatment. (A) Scheme of cytotoxicity assay performed using Tac-treated PP cells. (B) CCK-8 assay results of PP cells derived from iPSCs of patients with DM and non-DM individuals after incubation with different concentrations and for different exposure durations of Tac treatment. (C) Calculated area under the curve graphs from data in B. (D) Insulin messenger RNA (mRNA) levels in PP cells from patients with DM and non-DM individuals treated with 50 μg/mL Tac. Data are expressed as mean ± standard error. AUC, area under the curve; CCK-8, Cell Counting Kit-8; DM, diabetes mellitus; iPSC, induced pluripotent stem cell; KT, kidney transplantation; PP, pancreatic progenitor; Tac, tacrolimus.

*p < 0.05 vs. corresponding DM group.
in non-DM individuals, and iPSC-derived PP cells in the insulin generation-related system were more vulnerable in patients with DM than in non-DM individuals. Moreover, Tac toxicity screening showed a significant decrease in the number of PP cells of the DM group, suggesting that these cells are more susceptible to Tac toxicity. Therefore, our results revealed a genetic link between insufficient maturation of iPSCs into PP cells in the DM group.

We compared the reprogramming efficacy and pluripotency marker expression of the DM and non-DM groups. PBMCs were used as a platform for iPSC reprogramming because blood collection is less invasive than skin biopsy. For reprogramming, we used the Sendai virus transfection method. We did not detect any differences in reprogramming efficacy or pluripotency marker expression between patients with DM and non-DM individuals. Thus, iPSCs from patients with DM have similar pluripotent potential as cells from non-DM individuals.

A recent study examined clinical and genetic factors associated with new-onset DM after transplantation in a renal transplant population via a genome-wide association study [15]. The study revealed seven single nucleotide polymorphisms associated with genes implicated in the beta-cell apoptotic pathway, which may be the primary pathologic process in new-onset DM after transplantation [16,17]. We evaluated the transcriptomes of patient iPSCs using RNA-sequencing analysis to determine whether there were differences in genetic background between the DM and non-DM groups.

Using KEGG analysis, we performed functional and pathway enrichment analyses of the DEGs. Among the top 12 pathways, we focused on insulin resistance and type 2 DM, which included the DEGs SOCS3, MLXIPL, INSR, PPARA, PIK3R5, and SLC27A2. We also found that the TGF-β signaling pathway was strongly associated with iPSCs in the DM group. The in vitro protocol for differentiation of iPSCs into insulin-positive cells, including PP cells, involved inhibition of TGF-β signaling using a receptor antagonist, TGF-β R1 kinase inhibitor, and ALK5 inhibitor [7,8,11,18,19]. This suggests that downregulation of TGF-β signals is important during pancreatic development and beta-cell maturation. Taken together, these results indicate that genetic alterations in patients with DM are likely to play an important role in DM onset after KT.

Based on the above results, we evaluated the efficacy of PP cell differentiation in both groups. During differentiation into iPSC-PPs, we observed differences between the DM and non-DM groups in terms of differentiation efficiency. The iPSCs from the DM group consistently generated fewer PP cells than those from the non-DM group, and the mRNA levels of PP cell differentiation-related genes from both groups using qRT-PCR. The number of insulin-positive cells and insulin mRNA expression levels were significantly lower in patients with DM than in non-DM individuals. Confocal microscopy and EM showed decreased insulin expression in patients with DM. These findings suggest that patient-derived iPSCs exhibit defective differentiation of disease-related cells, and that PP cells show functional and morphologic defects in DM; therefore, PP cells can be used to predict DM.

Currently, there is no consensus regarding the preferred immunosuppression regimen to prevent DM, and individuals with DM are likely to be susceptible to immunosuppressive agents. Tac is the most popular treatment for preventing transplant rejection, but little is known about the methods for predicting beta cell injury in individuals. Therefore, we tested Tac-induced toxicity in iPSCs and PP cells and compared cell survival rates and insulin expression. The PP cells of the DM group showed significantly lower cell viability and insulin mRNA expression than those of the non-DM group; such differences were not observed for the iPSCs (Supplementary Fig. 2, Supplementary Methods, available online). These findings demonstrate that PP cells derived from patients with DM are more vulnerable to Tac toxicity than are those from non-DM individuals.

Our study has several limitations. First, it included only a few individuals per group, which is insufficient for representing overall new onset of DM population. Second, our study focused on Tac-induced DM; however, research involving other drugs, such as steroids, may also be required to evaluate DM. Third, potential target molecules need to be validated using inhibitors to confirm their functional roles.

In conclusion, we developed a novel approach for predicting DM before KT using patient-specific iPSCs. The model established in this study could be used to understand the pathophysiology of new-onset DM after KT, which may aid in developing novel therapeutics and predicting the risk of DM.
Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available on request from the corresponding author.

Authors’ contributions

Conceptualization, Data curation, Funding acquisition: SWL, CWY
Formal analysis: SWL
Investigation: SWL, YJS, SC, EJK, BHC
Methodology: YJS, SC, EJK, BHC
Writing–original draft: SWL, YJS, SC, EJK, BHC
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17. Hecking M, Haidinger M, Döller D, et al. Early basal insulin ther-


A 59-year-old man presented to our clinic with severe left flank pain after vigorous golfing. The patient exhibited tenderness at the left costovertebral angle. He had a history of hypertension and diabetes, both of which were well-controlled with medication. No recent trauma or endovascular procedures were reported. Laboratory tests showed elevated C-reactive protein (5.3 mg/dL) levels, but complete blood cell counts, creatinine levels, and urinalysis were unremarkable. A serum cholesterol test showed total cholesterol levels of 129 mg/dL, low-density lipoprotein cholesterol levels of 58 mg/dL, and triglyceride levels of 141 mg/dL. Computed tomography (CT) revealed a left renal infarction and the presence of dual left renal arteries with narrowing and irregularity in the left upper renal artery with an intraluminal hematoma, suggesting renal artery dissection. A 1.5-cm aneurysmal dilatation was also noted (Fig. 1A). CT angiography ruled out arterial occlusive disease, such as atherosclerosis, fibromuscular dysplasia, or connective tissue disorders (Fig. 2). Electrocardiogram and 24-hour Holter monitoring revealed normal sinus rhythm without arrhythmias. Echocardiography revealed the absence of intracardiac masses or thrombi. Laboratory tests revealed no abnormalities in coagulation. We suspected that spontaneous renal artery dissection (SRAD) caused the acute infarction in the left kidney. To prevent thrombosis at the site of endothelial damage and propagation of the dissection, anticoagulation was initiated with enoxaparin (1 mg/kg every 12 hours) and switched to oral warfarin. Oral warfarin therapy was continued for 6 months, aiming for an international normalized ratio range of 2 to 3. A follow-up CT scan revealed segmental atrophy of the left kidney, recovery of luminal flow in the dissected left upper renal artery, disappearance of the intraluminal thrombus, and regression of the aneurysm (Fig. 1B). Oral warfarin was discontinued and switched to low-dose aspirin as no thromboembolic risk factors were identified. No recurrence was noted during the 3-month follow-up period. Written informed consent was obtained from the patient for the publication of this report including all clinical images.

SRAD is a rare condition with few case reports on its occurrence after exercise. Renal infarction secondary to SRAD is even more uncommon. SRAD may occur during golfing owing to intimal tearing, which can be caused by direct stretching or acceleration and deceleration forces. SRAD should be considered in patients presenting with acute flank pain after strenuous exercise. Based on previous reports, we did not consider surgical or endovascular treatment as the patient’s blood pressure was effectively controlled and renal function remained stable under con-
Figure 1. CT images at initial presentation and 6-month follow-up with warfarin therapy. (A) Initial kidney CT scan shows luminal narrowing and irregularity of the left upper renal artery with intraluminal hematoma, suggesting renal artery dissection (yellow arrow, upper image) and a 1.5 cm aneurysmal dilatation proximal to the dissection site (orange arrow, lower image). (B) Follow-up CT scan after 6-months of warfarin therapy shows segmental atrophy of the infarcted site of the left kidney (red arrowheads), along with the recovery of luminal flow (yellow arrow, upper image), disappearance of the intraluminal thrombus (yellow arrow, upper image), and regression of the aneurysm (orange arrow, lower image) in the left upper renal artery.

CT, computed tomography.

Figure 2. CT angiography image. CT angiography shows no evidence of arteriopathies such as atherosclerosis, fibromuscular dysplasia, or connective tissue disorders in the aorta and its branches, including renal arteries.

CT, computed tomography.
servative treatment with anticoagulation. He achieved a good clinical outcome with medical treatment alone.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available on reasonable request to the corresponding author.

Authors’ contributions

Conceptualization: All authors
Data curation: SHJ, JHO, AYC, IOS, KYL, HL

Formal analysis: SHJ, HL
Visualization: SHJ, DMK, HL
Supervision: HL
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Rapidly progressive glomerulonephritis in the elderly: a case of cryoglobulinemic glomerulopathy not to be overlooked

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In very elderly patients (older than 80 years), the primary cause of rapidly progressive glomerulonephritis (RPGN) is pauci-immune necrotizing glomerulopathy, followed by anti-glomerular basement membrane (GBM) disease, amyloidosis, light-chain cast nephropathy, and focal segmental glomerulosclerosis [1]. Cryoglobulinemic glomerulopathy (CG), a subtype of monoclonal gammopathy of renal significance (MGRS), is a very rare kidney disease with unknown incidence and prevalence [2]. Type 1 CG mainly consists of monoclonal immunoglobulin (mIg), mostly IgM. It is associated with plasma cell disorders while type II and III CG are classified as mixed cryoglobulinemia, including both IgG and IgM [2,3]. MGRS is characterized by the proliferation of B lymphocytes or small plasma cell clones producing mlg and its components (light or heavy chains) [2,3]. Most kidney diseases associated with MGRS are glomerular disorders with light-chain proximal tubulopathy and crystal-storing histiocytosis [3] classified by mlg deposits’ characteristics on electron microscopy. MGRS is defined as nephrotoxic mlg deposition in the kidney that can lead to acute kidney injury [4,5]. Proactive etiological evaluation through kidney biopsy is required for MGRS presenting with RPGN features, as the treatment should be determined by the nature of the clone producing nephrotoxic mlg [6]. We report a case of MGRS associated with type I CG in an elderly patient with diabetic nephropathy demonstrating a distinct RPGN pattern.

An 86-year-old female patient was referred from a local clinic due to uncontrolled generalized edema. The patient’s significant medical history included 7 years of diabetes and hypertension, both managed with medication. She reported the use of diuretics prescribed at a private clinic for a month without achieving control of her edema. One month prior, her serum creatinine level was 1.07 mg/dL and her estimated glomerular filtration rate was 47 mL/min/1.73 m². She appeared to be in good health except for presenting with grade 4 edema in both legs. Upon admission, her serum creatinine had risen to 2.36 mg/
Table 1. Laboratory findings of the patient on admission

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.6</td>
<td>12.0–16.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>29.9</td>
<td>34.0–49.0</td>
</tr>
<tr>
<td>White blood cell (×10^6/L)</td>
<td>6,150</td>
<td>4,000–10,000</td>
</tr>
<tr>
<td>Platelet (×10^9/L)</td>
<td>184</td>
<td>150–450</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>47.4</td>
<td>6.0–20</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.36</td>
<td>0.5–0.9</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>140</td>
<td>136–145</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.7</td>
<td>3.5–5.1</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>108</td>
<td>98–110</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>7.7</td>
<td>8.6–10.2</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.1</td>
<td>6.6–8.7</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.6</td>
<td>3.5–5.2</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>107</td>
<td>35–104</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>23</td>
<td>0–32</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>15</td>
<td>0–33</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>242</td>
<td>0–250</td>
</tr>
<tr>
<td>Parathormone (pg/mL)</td>
<td>106</td>
<td>15–65</td>
</tr>
<tr>
<td>Vitamin D 25 OH (pg/mL)</td>
<td>20.89</td>
<td>19.9–79.3</td>
</tr>
<tr>
<td>IgG (mg/mL)</td>
<td>547</td>
<td>700–1600</td>
</tr>
<tr>
<td>IgM (mg/mL)</td>
<td>102</td>
<td>40–230</td>
</tr>
<tr>
<td>IgA (mg/mL)</td>
<td>244</td>
<td>70–400</td>
</tr>
<tr>
<td>Kappa lambda ratio</td>
<td>0.63</td>
<td>0.26–1.65</td>
</tr>
</tbody>
</table>

(\(\text{Table 1}\)). Initially, her albumin/globulin (A/G) ratio showed a reversal at 0.89. Serum electrophoresis did not show a monoclonal peak with increases in alpha 1/2 and beta 2 fractions (\(\text{Fig. 1A}\)). Serum immuno-fixation electrophoresis also showed a normal immune typing pattern. Her serum cryoglobulin was negative. Autoimmune tests were negative for anti-GBM antibody (Ab), anti-proteinase 3 Ab, and anti-myeloperoxidase Ab. Despite treating the edema with diuretics, there was no improvement. Renal ultrasound and computed tomography revealed decreased sizes of both kidneys with echogenicity consistent with renal parenchymal diseases (\(\text{Fig. 1C}\)). Kidney biopsy was delayed due to the patient’s advanced age, existing diabetic kidney disease, and aspirin use. However, as there was no response to steroid treatment and RPGN was clinically suspected, a kidney biopsy was eventually performed. The kidney biopsy revealed type I CG combined with diabetic nephropathy that was consistent with membranoproliferative glomerulonephritis characterized by increased mesangial matrix and cellularity having lobular accentuation and hyaline intraluminal thrombi (\(\text{Fig. 1D}\)). Immunofluorescence microscopy showed intraluminal staining for Ig lambda (\(\text{Fig. 1E}\)), but not kappa (not shown). There also was intraluminal staining for C1q and a predominance of IgM (\(\text{Figs. 1F, G, respectively}\)). Electron microscopy revealed that some tubular basement membranes were thickened and multilayered. The interstitium exhibited edema and infiltration by inflammatory cells (\(\text{Figs. 1H, I}\)). Bone marrow biopsy demonstrated no evidence of monoclonal hematologic disease (\(\text{Fig. 1J}\)). Since initiating continuous renal replacement therapy (CRRT) due to her rapid deterioration of renal function with metabolic acidosis and oliguria, the patient’s condition continued to worsen throughout her admission.

Despite treatment with CRRT, plasmapheresis, and pulse methylprednisolone, her renal function, hematologic anomalies, and systemic condition deteriorated further (\(\text{Fig. 1B}\)). This report was approved from the Institutional Review Board of the Catholic University of Korea, Seoul St. Mary’s Hospital (No. KC23ZASI0826). Written informed consent was obtained from the patient for the publication of this report including all clinical images.

For MGRS-related diseases, clone-directed therapy such as bortezomib and ofatumumab, which were not prescribed in this patient, would have resulted in better outcomes than high-dose prednisolone treatment [6]. In this report, we diagnosed MGRS with type 1 CG presenting with intractable edema, a reversed serum A/G ratio, and a full-fledged nephrotic syndrome, which led to renal failure and ultimately the patient’s death. Recognizing MGRS is crucial for managing end-organ damage and improving patient survival, potentially leading to better treatments with fewer adverse effects, even in very old patients [1]. Early diagnosis is key to successfully treating RPGN, including MGRS. However, this can be particularly challenging in the elderly, especially for those with hypertension and diabetes as these patients often present with typical or minimal clinical symptoms [7]. The diagnosis may be challenging because the hallmark of cryoglobulinemia is the detection of cryoglobulins in the serum. However, cases of CG without serological evidence of cryoglobulinemia are often diagnosed by anatomicopathological findings in renal biopsy [8].

In conclusion, our case report emphasizes the importance of considering MGRS as a potential cause of RPGN in elderly patients, even if they have underlying renal diseas-
Figure 1. Comprehensive clinical and pathological profile of the patient. (A) Serum electrophoresis does not show a monoclonal peak. (B) The clinical course of the patient. (C) Abdominal computed tomography scan shows decreased sizes of both kidneys (right, 9.6 × 5.6 cm and left, 9.0 × 4.5 cm) with irregular contours, indicating chronic renal parenchymal disease. Kidney biopsy reveals (D) type I cryoglobulinemic glomerulopathy combined with diabetic nephropathy, consistent with membranous proliferative glomerulonephritis characterized by increased mesangial matrix and cellularity having lobular accentuation and hyaline intraluminal thrombi (×400). (E) Immunofluorescence microscopy (×400) shows intraluminal staining for immunoglobulin lambda. (F) Intraluminal staining for C1q (×400). (G) Predominant IgM is observed (×400). (H, I) Electron microscopy (×6,000) reveals thickened and multilayered tubular basement membranes (I, arrow). The interstitium shows edema and infiltration of inflammatory cells. (J) CD138-stained bone marrow slide (×20) exhibits a hypo-cellular pattern with plasma cells accounting for approximately 3% of all nucleated elements, slightly elevated compared to the normal range of 0% to 1%. However, it does not meet the criteria for plasma cell myeloma or other B cell/plasma cell proliferative disorders. No evidence of amyloidosis or kappa, lambda restriction was found in the bone marrow examination.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Quant (g/dL)</th>
<th>Reference (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>4.9</td>
<td>6.6–8.3</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>2.30</td>
<td>4.02–4.76</td>
</tr>
<tr>
<td>α1</td>
<td>0.26</td>
<td>0.21–0.35</td>
</tr>
<tr>
<td>α2</td>
<td>1.06</td>
<td>0.51–0.85</td>
</tr>
<tr>
<td>β1</td>
<td>0.29</td>
<td>0.34–0.52</td>
</tr>
<tr>
<td>β2</td>
<td>0.42</td>
<td>0.23–0.47</td>
</tr>
<tr>
<td>γ</td>
<td>0.57</td>
<td>0.80–1.35</td>
</tr>
<tr>
<td>Monoclonal peak</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

It underscores the significance of conducting a kidney biopsy to accurately determine the cause of kidney injury, thereby facilitating proper and timely treatment.

Conflicts of interest

All authors have no conflicts of interest to declare.

Data sharing statement

The data presented in this study are available upon reasonable request to the corresponding author.

Authors’ contributions

Conceptualization, Data curation: HP, CWP
Methodology: SR, SYC, EAK, JMK, YK, YJC
Writing–original draft: HP
Writing–review & editing: All authors
All authors read and approved the final manuscript.

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References

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이 약의 적절한 용량은 각 환자에 따라 주의 깊게 결정되어야 한다. 만성신부전 환자에서 현재 인정되는 완전한 부갑상샘호르몬 (intact PTH) 수치의 목표 범위는 요독증이 없는 정상치 상한의 1.5~3 배보다 높지 않다. 이 약의 권장 초기 용량은 2일 1회 또는 이보다 반반하지 않은 빈도로 투석 시 0.04~0.1 μg/kg(2.8~7 μg)을 일시 주사한다.(상세 내용은 제품 설명서 참조)

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Ref.) 제품 허가사항, 식약처 의약품안전나라, accessed on 2022.06.20

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2. 렌ベル라®정 국내허가사항 (as of 2023-08-08)

CKD, chronic kidney disease; Hb, hemoglobin

References
1. 미쎄라® 프리필드주 국내허가사항 (as of 2023-08-08)
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