Close linkage between serum uric acid and cardiac dysfunction in patients with ischemic heart disease according to covariance structure analysis

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High serum uric acid (UA) level has been assumed to be a risk factor for left ventricular (LV) dysfunction; however, the precise relationship between these conditions has not been fully examined because many confounding factors are associated with UA level. We herein examined the precise relationship by proposing structural equation models. The study population consisted of 1432 cases with ischemic heart disease who underwent cardiac catheterization. Multiple regression analyses and covariance structure analyses were performed to elucidate the cause-and-effect relationship between UA level and LV ejection fraction (LVEF). A path model exploring the factors contributing to LVEF showed that high UA was a significant cause of reduced LVEF ($P = 0.004$), independent of other significant factors. The degree of atherosclerosis, as estimated by the number of diseased coronary vessels, was significantly affected by high UA ($P = 0.005$); and the number of diseased coronary vessels subsequently led to reduced LVEF ($P < 0.001$). Another path model exploring the factors contributing to UA level showed that LVEF was a significant cause of high UA ($P = 0.001$), while other risk factors were also independent contributing factors. This study clearly demonstrated that there was a close link between high UA and LV dysfunction, which was represented by possible cause-and-effect relationship.

Patients with high uric acid (UA) levels are predisposed to gout as a major clinical complication. With the increased awareness of the hazardousness of high UA levels, discussion has also recently resurfaced in the cardiovascular field$^{1-3}$. According to epidemiological research and meta-analyses, the serum UA level predicts the progression of chronic kidney disease and the development of stroke$^{4,5}$. Furthermore, elevated UA levels are associated with the presence of hypertension, diabetes, and metabolic syndrome$^{6-8}$, while the relationship between ischemic heart disease (IHD) and serum UA level remains controversial. Another recent meta-analysis studying the relationship between serum UA and IHD showed that a high UA level was not likely to be a main determinant of IHD and that it may not significantly contribute to the prediction of IHD in the general population$^9$.

In contrast, in patients with heart failure (rather than IHD), there has been increasing evidence to indicate that high UA levels predict an increase in morbidity and mortality$^{2,10}$. Nonetheless, few studies have shown a precise relationship between high UA levels and cardiac function, as measured in detail by echocardiography, cardiac catheterization, and other methods. Thus, the direct relationship between cardiac systolic dysfunction and high UA levels remains unclear and an investigation should be performed in which the effects of high UA levels on the process of coronary atherosclerosis are considered.

When taken together, although it has almost been accepted that high UA levels are associated with most cardiovascular diseases, including heart failure, many more studies are required to confirm the positioning of high UA level as a risk factor for the respective cardiovascular disorders. A statistical analysis would be helpful; however,
there is a degree of intractableness associated with performing a precise analysis because serum UA level is likely to be associated with many other risk factors, including but not limited to male gender, obesity, dyslipidemia. All of these risk factors may—both individually and collectively—affect the progression of cardiovascular diseases. It therefore seems quite difficult to confirm the risk that a high UA level itself poses in relation to cardiovascular diseases. Advanced statistical methods will be needed in order to elucidate the precise risk of high UA levels.

In a statistical analysis, confounders are extraneous variables that affect the variables being studied—when confounders are present, the results do not reflect the actual relationships between the studied variables. Confounding variables are often defined as variables that are correlated—either positively or negatively—with both the dependent variable and the independent variable. There are several ways to eliminate confounding bias, for example, by adjustment of the independent variables. Still, a confounding bias can be difficult to control if multiple potential confounding variables are present or if the study population is of insufficient size. A covariance structure analysis plays an important role in understanding how the relationships between observed variables may be generated in many areas using hypothesized latent variables. A covariance structure analysis is useful for exploratory and explanatory factor analyses. This analysis can be performed based on the confounding bias. Another merit of using a covariance structure analysis is that the analysis can package Bayesian networks to infer cause-and-effect relationships. However, the factors that are included in these analyses should be carefully selected, and the path model based on covariance structure analysis should be proposed based on consistent concepts and the clear direction of the study. In a tangible way, we successfully proposed a path model based on a covariance structure analysis in order to explain a complex phenomenon involving possible causality.

In the present study, we examined the effects of high UA levels on cardiac function and the cause-and-effect relationship between them using cardiovascular disease patients, all of whom underwent cardiac catheterization in our institution. We performed a step-by-step statistical analysis to identify the risk of high UA per se for cardiovascular dysfunction while taking into account the influence of high UA levels on IHD—as evaluated by organic stenosis in the coronary arteries and the effect of IHD on the cardiac function.

**Results**

**The characteristics of the study patients.** The clinical characteristics of the 1432 cases are shown in Table 1. The mean UA level was 6.1 ± 1.4 mg/dL and the mean left ventricular ejection fraction (LVEF) was 58.7 ± 10.5%.

**The multiple regression analysis to determine the association between risk factors, LVEF and the number of diseased vessels.** A multiple regression analysis was performed to examine the effects of risk factors on the LVEF (Supplementary Table S1). The analysis revealed that age (P < 0.001), body mass index (BMI) (P < 0.001), estimated glomerular filtration rate (eGFR) (P < 0.001), triglyceride (TG) (P = 0.002), systolic blood pressure (AOsys) (P < 0.001), hemoglobin A1c (HbA1c) (P < 0.001), smoking habit (P = 0.030), and UA (P < 0.001) were significant factors. Another multiple regression analysis was performed to examine the effects of risk factors on the number of diseased vessels (Supplementary Table S2). The analysis identified age (P = 0.015), eGFR (P = 0.017), TG (P = 0.033), AOsys (P = 0.001), HbA1c (P < 0.001), and UA (P = 0.011) as significant factors.

**The multiple regression analysis to determine the factors associated with serum UA level.** A multiple regression analysis was performed to examine the effect of risk factors on the serum UA level (Supplementary Table S3). The analysis identified age (P = 0.002), gender (P < 0.001), BMI (P < 0.001), eGFR (P < 0.001), TG (P < 0.001), AOsys (P = 0.017), HbA1c (P = 0.018), and LVEF (P = 0.002) as significant factors.

**The single regression analysis to search for confounding biases among the risk factors.** A single regression analysis was performed in order to search for confounding biases among the independent variables. Most of the pairs showed a significant association (Supplementary Table S4). Thus, the results of the above-mentioned multiple regression analyses lost some of their meaning.

**The concept of the proposed path model (A): A high UA level as a possible cause of LV dysfunction.** As a matter of logic, the theoretical path model (A) was proposed by positioning the serum UA level in parallel with the other risk factors of age, gender, BMI, TG, smoking, AOsys, eGFR, and HbA1c (Fig. 1). In order to examine possible causality, paths between variables were drawn from independent variables to dependent variables with a directional arrow for each regression model. All of the risk factors had the potential to confound each other. The association between two factors was linked by two-way arrows. In this path model, the number of vessel diseases was positioned to convert a path from all of the risk factors to LVEF because the degree of coronary artery disease is possibly influenced by all risk factors and then subsequently affects LVEF.

**The results of the path model (A).** The precise results of the path model (A) are shown in Table 2 and Supplementary Table S5. The exploratory factor analysis revealed that age, BMI, TG, AOsys, eGFR, HbA1c, and UA were significant causes of reduced LVEF. The negative correlation between UA level and LVEF was found with a significant impact (standardized regression coefficients, β = −0.086, P = 0.004). Furthermore, the exploratory factor analysis revealed that age, BMI, TG, AOsys, eGFR, HbA1c, and UA were significant causes of the number of diseased vessels. The number of diseased vessels was causally linked to a reduction of LVEF.

**The concept of proposed path models (B) and (C): LV dysfunction as a possible cause of high UA level.** The theoretical path model (B) was proposed by positioning LVEF in parallel with the risk factors of age, gender, BMI, TG, AOsys, smoking, eGFR, and HbA1c, all of which possibly affect the serum UA level (Fig. 2). The serum UA level may also be affected by the medication profile, such as the usage of UA lowering agents and...
diuretics. Thus, another theoretical path model (C) was proposed (Supplementary Fig. S1). All of the risk factors had the potential to confound each other. The association between two factors was linked by two-way arrows. The results of path models (B) and (C).

The precise results of path models (B) and (C) are shown in Tables 3 (and Supplementary Table S6) and Supplementary Table S7, respectively. The exploratory factor analysis revealed that age, gender, BMI, TG, AOSys, eGFR, HbA1c, and LVEF were significant causes of high serum UA levels (Table 3). Moreover, although the usage of diuretics were significant factors that affected the serum UA level, LVEF was consistently negatively correlated with the serum UA level independently of the medication profile (Supplementary Table S7).

Discussion

It has recently been reported that a high UA level is a significant risk factor for many cardiovascular diseases, including heart failure; however, the precise positioning of UA in these conditions remains unclear and controversial behind the high impact of metabolic syndrome. One of the difficulties in research the impact of UA is probably attributable to the intractableness of the statistical approach because the serum UA levels are tightly

| Characteristics (n = 1432) | Overall; Number (%) or Mean ± SD [Median; range] |
|---------------------------|--------------------------------------------------|
| Gender; Male/Female       | 1235/197 (86.2/13.8)                             |
| Age (years old)           | 66.5 ± 11.0                                     |
| BMI (kg/m²)               | 24.7 ± 3.8                                      |
| Current smoker            | 278 (19.4)                                      |
| Family history of IHD     | 367 (25.6)                                      |
| Hb (g/dL)                 | 13.4 ± 1.8                                      |
| Cr (mg/dL)                | 0.93 ± 0.53                                     |
| eGFR (mL/min/1.73 m²)     | 68.6 ± 19.3                                     |
| UA (mg/dL)                | 6.1 ± 1.4                                       |
| FBS (mg/dL)               | 118 ± 31.5                                      |
| HbA1c (%)                 | 6.3 ± 1.0                                       |
| TG (mg/dL)                | 127.5 ± 102.3                                   |
| HDL-C (mg/dL)             | 51.3 ± 14.7                                     |
| LDL-C (mg/dL)             | 97.4 ± 26.6                                     |
| LDL-C/HDL-C               | 2.04 ± 0.78                                     |
| CRP (mg/dL)               | 0.39 ± 1.23                                     |
| BNP (pg/mL)               | 94.8 ± 187.0 [35.7; 3.0–2520.5]                 |
| LVEF (%)                  | 58.7 ± 10.5                                     |

| Underlying cardiovascular disease number |
|------------------------------------------|
| Cardiomyopathy                           | 32 (2.2)                                       |
| Valvular disease                         | 60 (4.2)                                       |
| Atrial fibrillation                      | 60 (4.2)                                       |
| Hypertension                             | 1112 (77.7)                                    |
| Diabetes mellitus                        | 617 (43.1)                                     |
| Dyslipidemia                             | 1135 (79.3)                                    |
| Renal dysfunction*                       | 412 (28.8)                                     |

| Medication                              |
|------------------------------------------|
| ACE inhibitors                           | 330 (23.0)                                     |
| ARBs                                     | 633 (44.2)                                     |
| Beta blockers                            | 665 (46.4)                                     |
| Calcium channel blockers                 | 879 (61.4)                                     |
| Diuretics                                | 269 (18.8)                                     |
| Statins                                  | 1023 (71.4)                                    |
| Non-Statin for dyslipidemia              | 194 (13.5)                                     |
| Oral antidiabetic agents                 | 442 (30.9)                                     |
| Insulin                                  | 163 (11.4)                                     |
| UA lowering agents                       | 246 (17.2)                                     |

Table 1. Clinical characteristics. BMI, body mass index; IHD, ischemic heart disease; Hb, hemoglobin; Cr, Creatinine; eGFR, estimated glomerular filtration rate; UA, uric acid; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; TG, triglycerides; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; CRP, C-reactive protein; BNP, B-type natriuretic peptide; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; and ARBs, angiotensin II type I-receptor blockers. *Renal dysfunction = eGFR < 60 mL/min/1.73 m² (excluded hemodialysis-dependent patients).
associated with numerous other risk factors. In this study, we used a covariance structure analysis together with single and multiple regression analyses. A covariance structure analysis can eliminate confounding bias and clarify possible cause-and-effect relationships. As a result, we found that a high UA level was causally related to LV dysfunction and vice versa, suggesting that there is a possible cause-and-effect linkage between these factors.

The current study showed that high UA levels were thought to be causally reduced LVEF. In this study, we took account of the severity of IHD because IHD is affected by numerous risk factors, and subsequently affects LV dysfunction. The path model (A) was proposed in order to simultaneously estimate the effects of risk factors for

Table 2. The results of path model A. The results (direct, indirect, and total effects) of the path model theoretically proposed analysis to identify the clinical factors influencing between each other (see Fig. 1).

| Clinical Factor | Estimate | Standard error | Test statistic | P-Value | Standard regression coefficient |
|-----------------|----------|----------------|----------------|---------|---------------------------------|
| Vessel Disease  |          |                |                |         |                                 |
| (R² = 0.076)    |          |                |                |         |                                 |
| Age             | 0.006    | 0.003          | 2.342          | 0.019   | 0.074                           |
| Gender          | 0.088    | 0.077          | 1.153          | 0.249   | 0.165                           |
| BMI             | -0.014   | 0.007          | -2.057         | 0.040   | -0.058                          |
| TG              | -0.001   | 0.000          | -2.319         | 0.020   | -0.063                          |
| Smoking         | 0.058    | 0.037          | 1.573          | 0.116   | 0.043                           |
| AO sys          | 0.004    | 0.001          | 5.502          | <0.001  | 0.101                           |
| eGFR            | -0.004   | 0.002          | -2.725         | 0.006   | -0.086                          |
| HbA1c           | 0.161    | 0.026          | 6.142          | <0.001  | 0.165                           |
| UA              | 0.055    | 0.020          | 2.792          | 0.005   | 0.080                           |
| LVEF            |          |                |                |         |                                 |
| (R² = 0.146)    |          |                |                |         |                                 |
| Age             | 0.176    | 0.032          | 5.500          | <0.001  | 0.183                           |
| Gender          | 0.088    | 0.085          | -1.887         | 0.059   | -0.055                          |
| BMI             | -0.014   | 0.080          | 3.887          | <0.001  | 0.011                           |
| TG              | -0.001   | 0.003          | 2.502          | 0.012   | 0.071                           |
| Smoking         | -0.810   | 0.429          | -1.889         | 0.059   | -0.054                          |
| AO sys          | 0.057    | 0.012          | 4.548          | <0.001  | 0.131                           |
| eGFR            | 0.107    | 0.018          | 5.935          | <0.001  | 0.195                           |
| HbA1c           | -0.106   | 0.305          | -3.458         | <0.001  | -0.098                          |
| UA              | -0.652   | 0.227          | -2.868         | 0.004   | -0.086                          |
| Vessel Disease  | -2.417   | 0.306          | -7.797         | <0.001  | -0.219                          |

Figure 1. The path model [A]: An explanatory drawing of the possible cascade from risk factors to the number of diseased vessels and LVEF. This path has a coefficient showing the standardized coefficient of a regressing independent variable on a dependent variable of the relevant path. These variables indicate standardized regression coefficients (direct effect) [bold typeface indicates remarkable values], squared multiple correlations [narrow italics] and correlations among exogenous variables [green]. BMI, body mass index; TG, triglyceride; AO sys, systolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; UA, uric acid; e, extraneous variable.
both IHD and LVEF in a single equation model. This analysis indicated that high UA levels were causally linked to the severity of IHD and then that IHD affected LVEF. Importantly, it was clarified that high UA levels reduced LVEF independently of the severity of IHD.

We found that an older age, male gender, obesity, hypertriglyceridemia, hypertension, renal dysfunction, and diabetic condition were major factors associated with high UA levels in Path model B. The current results are mostly in agreement with those of previous reports; hyperuricemia is closely associated with visceral fat accumulation\cite{12,13} and various metabolic disorders, such as glucose intolerance, elevated blood pressure, dyslipidemia, and atherosclerotic cardiovascular diseases, which are conceptualized as metabolic syndrome\cite{14-18}. However, smoking fell short of being significantly associated with hyperuricemia, in contrast to the findings in previous reports\cite{19,20}.

In this study, 85.5% of the patients were male and 14.7% were female. Generally, the serum UA level is elevated during puberty in men and after menopause in women. In addition, the UA clearance in the kidney is low in men.

Table 3. The results of path model B. The results (direct effect) of the path model theoretically proposed analysis to identify the clinical factors influencing between each other (see Fig. 2). RMSEA 0.133, AIC 130.0. \(R^2\): squared multiple correlations. UA, uric acid; BMI, body mass index; TG, triglycerides; AO sys, Systolic blood pressure in the Aorta; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LVEF, left ventricular ejection fraction.
Thereby, there is a gender difference in the serum UA level, which might have influenced the results of the current study. However, given the small number of female patients in the study, we are unable to make a conclusive decision at present, and analyses concerning gender differences in serum UA levels should be performed in the future.

Although the molecular mechanisms underlying the harmful effects of high UA levels have not been fully examined, the activation of redox-dependent effects, extracellular regulated kinase (ERK), proinflammatory pathway, and endothelin-1 are likely involved. Furthermore, elevated UA levels reflect upregulated xanthine oxidase (XO) activity, which induces oxidative stress. These findings suggest that a high UA level is a major risk factor for the development of cardiovascular diseases. On the other hand, this study also indicated that LV dysfunction causally induced high UA levels. The molecular mechanisms are also unclear at present; however, the proposed mechanisms are as follows: adenosine triphosphate (ATP), which is synthesized in the mitochondria, is transferred to myofibril by phosphocreatine (PCr) through the Cr kinase energy shuttle, and is used by the contractile mechanism to form adenosine diphosphate (ADP), which is re synthesized by oxidative phosphorylation in the mitochondria. In states of ATP depletion and reduced Cr kinase shuttle, such as heart failure, the metabolic turnover of the purine metabolism is increased, and the breakdown paths below ADP are activated, eventually reaching the breakdown endpoint of UA by the activation of XO. At present, it is unclear whether this biological reaction occurs in the cardiomyocytes. Future research should be focused on the precise energetic metabolic changes that occur in connection with high UA levels in an in vitro analysis.

When taken together, this study suggests that high UA levels lead to the development of heart failure; and vice versa (namely, that heart failure induces high UA levels), drifting into a vicious cycle. The current study strongly supports the previous reports that show the significance of high UA levels as a harmful effect in heart failure. Overall, the present findings indicate that UA is not only an active player but also an indirect marker strongly supports the previous reports that show the significance of high UA levels as a harmful effect in heart failure, drifting into a vicious cycle. The current study in vitro energetic metabolic changes that occur in connection with high UA levels in an in vitro analysis.

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Methods

Study patients. The study population consisted of 1432 cases with IHD who were consecutively admitted to our institutions from 2012 to 2016. All of the patients underwent cardiac catheterization for an evaluation of IHD. We excluded the patients who underwent hemodialysis because their cardiac function was significantly altered by artificial volume control. Emergency cases (i.e. acute coronary syndrome) were also excluded, as their hemodynamics (including LVEF) and miscellaneous biomarkers are highly variable and do not reflect the values under stable conditions. The ethics committee of The Jikei University School of Medicine approved the study protocol (24–355[7121]), and we complied with the routine ethical regulations of our institution. This was a retrospective study, and informed consent could not be obtained from each patient. Instead of obtaining informed consent from each patient, we posted a notice about the study design and contact information at a public location in our institution.

The disease definitions. IHD was diagnosed based on symptoms, electrocardiography (ECG), blood sampling, and the coronary artery morphology. Organic lesions producing ≥75% luminal stenosis of the coronary arteries on coronary angiography were defined based on the modified American Heart Association (AHA) coronary tree segment classification48. The number of diseased vessels was counted as the number of three major coronary arteries (i.e. left anterior descending [LAD], left circumflex [LCx] and right coronary arteries) with organic lesions that were indicated for treatment by revascularization and/or standard medical therapy. Diagonal and high lateral branches were included in LAD and LCx, respectively, if they had a substantial myocardial perfusion area and were indicated for treatment. In the present study, we divided the patients into four groups based on the number of diseased vessels with significant organic stenosis (the 0-, 1-, 2-, and 3-vessel groups). Patients with a left main trunk lesion were included in the 2-vessel group. The number of diseased vessels was counted at the time of cardiac catheterization in this study, and lesions already treated by revascularization were not included. Patients with coronary spasm (diagnosed on the basis of clinical findings, including ECG change, or a provocation test with intracoronary injection of acetylcholine) were placed into the 0-vessel group if there was no organic stenosis after nitroglycerin administration. Some of the patients had comorbid cardiovascular diseases, such as valvular disease, arrhythmia, cardiomyopathy, and other conditions. Hypertension, Diabetes mellitus (DM) and dyslipidemia were defined as described previously49. The eGFR was calculated according to the Modification of Diet in Renal Disease (MDRD) Study equation shown below40, with coefficients modified for Japanese patients41.

\[
eGFR (\text{ml/min/1.73m}^2) = 194 \times \text{age}^{-0.287} \times \text{Creatinine}^{-1.094} \times (\times 0.739 \text{for females})
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Renal dysfunction was defined by an eGFR of <60 ml/min/1.73 m², according to the guidelines of the Japanese Society of Nephrology.

Blood sampling and hemodynamic examinations during cardiac catheterization. We collected blood samples and hemodynamic data during cardiac catheterization. The serum biochemical analyses were performed in a central laboratory of our hospital during the study period. In this statistical analysis, we included serum levels of Cr, UA, and TG, AOsys, and HbA1c level. We included smoking habit (0 for non-smoker, 1 for past smoker, and 2 for current smoker). LVEF was measured at the time of left ventriculography42.

Statistical analysis. Continuous variables are expressed as the mean ± standard deviation (SD) or medians. The correlation between two factors was investigated by a single regression analysis and expressed as Spearman’s correlation coefficient. A multiple regression analysis was performed to compare multiple values. The above-mentioned statistical analyses were performed using the SPSS Statistics software program (version 23.0, SPSS Inc., Chicago, IL, USA). P values of <0.05 were considered to indicate statistical significance.

A path analysis based on a covariance structure analysis was used to investigate the relationship between clinical factors in this study population and to survey the probable causal effects on LVEF or serum UA level. The path analysis was performed using the IBM SPSS AMOS software program (version 23, Amos Development Corporation, Meadville, PA, USA). We have previously described how to write a path model41. In brief, the possible causality model defined some hierarchical regression models between clinical factors and LVEF or serum UA level. For every regression, the total variance in dependent variable is theorized to be caused by either independent variables that are included in the model or by extraneous variables (e). The indirect effect was determined by levels. For every regression, the total variance in dependent variable is theorized to be caused by either independent

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Blood sampling and hemodynamic examinations during cardiac catheterization. We collected blood samples and hemodynamic data during cardiac catheterization. The serum biochemical analyses were performed in a central laboratory of our hospital during the study period. In this statistical analysis, we included serum levels of Cr, UA, and TG, AOsys, and HbA1c level. We included smoking habit (0 for non-smoker, 1 for past smoker, and 2 for current smoker). LVEF was measured at the time of left ventriculography42.

Statistical analysis. Continuous variables are expressed as the mean ± standard deviation (SD) or medians. The correlation between two factors was investigated by a single regression analysis and expressed as Spearman’s correlation coefficient. A multiple regression analysis was performed to compare multiple values. The above-mentioned statistical analyses were performed using the SPSS Statistics software program (version 23.0, SPSS Inc., Chicago, IL, USA). P values of <0.05 were considered to indicate statistical significance.

A path analysis based on a covariance structure analysis was used to investigate the relationship between clinical factors in this study population and to survey the probable causal effects on LVEF or serum UA level. The path analysis was performed using the IBM SPSS AMOS software program (version 23, Amos Development Corporation, Meadville, PA, USA). We have previously described how to write a path model41. In brief, the possible causality model defined some hierarchical regression models between clinical factors and LVEF or serum UA level. For every regression, the total variance in dependent variable is theorized to be caused by either independent variables that are included in the model or by extraneous variables (e). The indirect effect was determined by multiplying the path coefficients of the intervening variables. The structural equation models that were obtained were tested and confirmed; P values of <0.05 were considered to indicate statistical significance.

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**Author Contributions**

Y.T. collected the data, performed the statistical analyses, and wrote the manuscript. T.N. and M.Y. conceived of the research hypothesis and analyses, wrote and edited the manuscript. M.K., G.U., S.I., and K.M. performed the statistical analyses and edited the manuscript. A.Y., H.K., Y.I., K.O., T.T. and T.O. participated in the design and coordination of the study and collected the data. All authors read and approved the final manuscript.

**Additional Information**

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