Online Supplement

MATERIALS AND METHODS

Study design and patients

The present study is part of an ongoing investigation aimed at prospectively examining the role of ionized magnesium (Mg^{2+}) in the clinical outcomes of hemodialysis patients. Patients were enrolled during a one-year period, from August 2021 to July 2022 (the Mg^{2+} monitoring period), and followed up until the end of September 2024. The inclusion criteria were regular maintenance hemodialysis at the Kidney Center of our institute and the presence of baseline Mg^{2+} measurement during the Mg^{2+} monitoring period. The exclusion criteria included scheduled major surgery or scheduled transfer within one month at the time of attempted enrollment. During the Mg^{2+} monitoring period, Mg^{2+} and total serum magnesium (tsMg) were added to routine biochemical examinations, which were conducted at midday of the first week of the month for outpatients and the third week for inpatients. Patients received hemodialysis three times a week and medical treatment following the guidelines of Japanese Society of Nephrology and the Japanese Society for Dialysis Therapy.

Blood biochemistry analysis

Blood samples collected immediately before hemodialysis were used for blood and serum biochemical analyses. Blood pH, Mg^{2+}, sodium, potassium, chloride, and ionized calcium were determined with a Stat Profile Prime ES Comp Plus (NOVA Biomedical Co., Waltham, MA, USA), which is equipped with highly sensitive ion-selective electrodes. Blood Mg^{2+} levels obtained via this method have been shown to be virtually identical to those measured from serum samples [1]. An AU480 Chemistry Analyzer and a UniCel Dx600 Hematology Analyzer (Beckman Coulter Inc., Brea, CA, USA) were used to measure serum biochemical and hematological parameters. The corrected serum calcium levels (c-calcium) were calculated by Payne's formula, c-calcium (mg/dL) = serum calcium (mg/dL) + (4.0 -serum albumin (g/dL)).

For the diagnosis of Mg^{2+}- and tsMg-defined dysmagnesemia, we employed recently published 95% reference ranges for Mg^{2+} and tsMg levels in healthy adult population, which are 0.40 – 0.68 mmol/L and 0.72 – 1.00 mmol/L, respectively [2].

Primary and secondary endpoints

The primary endpoint was the occurrence of death from all causes. The secondary endpoint was cardiovascular mortality, which was defined as death caused by myocardial infarction, heart failure, stroke, arrhythmia, or sudden death.

Statistical analyses

Numerical data are presented as the mean ± standard deviation (SD) or median (interquartile range), depending on the distribution. Categorical variables are expressed as numbers and frequencies (percentages). Patients were divided into three groups on the basis of tertiles of Mg^{2+} or tsMg at baseline. Differences in clinical characteristics and biochemical data among the three groups were evaluated using analysis of variance (ANOVA), the Kruskal-Wallis test, or the χ−square test, as appropriate. Pairwise comparisons for parameters showing differences between the tertile groups were conducted by Turkey-Krammer HSD test, the Steel-Dwass test or pairwise χ−square tests with Bonferroni correction.

Associations of demographic and biochemical parameters with the primary and secondary endpoints were analyzed using univariate and multivariate Cox regression analyses and restricted mean survival time (RMST) analysis [3, 4]. Explanatory variables were selected on the basis of clinical relevance and findings of earlier studies, with the aim of minimizing the corrected Akaike information criterion (AICc) and Bayesian information criterion (BIC) of the models. Among the 249 patients included in the analysis, 33 patients were transferred to other dialysis facilities (n = 32) or received renal transplantation (n = 1) during the follow-up period and were therefore censored at the time of transfer in the survival analyses. Relationships between magnesium level tertiles and patient survival were also assessed via Kaplan-Meier analysis with the log-rank test. Because Kaplan–Meier analysis results raised concern regarding the proportional hazards assumption, RMST analysis was performed to evaluate survival differences among the tertile groups.

To validate the findings from the Cox regression analyses for the primary endpoint, three sensitivity analyses were conducted. First, continuous values of Mg^{2+} were used instead of tertiles of Mg^{2+} for Cox analysis. Second, patients on magnesium supplements at baseline (n = 23) were excluded, and the regression analysis was repeated for the remaining 226 patients. Third, logistic regression analysis for the primary endpoint was conducted by using data for all patients.

To identify factors affecting Mg^{2+} and tsMg levels, multiple regression analyses were performed using demographic and laboratory variables. Explanatory variables for the models were selected from demographic and laboratory parameters via the forward stepwise method.

All statistical tests were two-tailed, and a p value of <0.05 was considered statistically significant. Statistical analyses were performed using JMP Pro 17 (JMP Statistical Discovery LLC, Cary, NC, USA). Stata (StataCorp LLC, College Station, TX, USA) was also used for restricted cubic spline analysis. A p value of <0.05 was considered to indicate statistical significance.

References

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