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Comparative Analysis of Liquid Chromatography-Tandem Mass Spectrometry and Radioimmunoassay in Determining Plasma Aldosterone Concentration and Plasma Renin Activity for Primary Aldosteronism Screening

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) accurately measures plasma aldosterone concentration (PAC), but its correlation with radioimmunoassay (RIA), equivalent RIA levels, and optimal cutoff for PAC and aldosterone-to-renin ratio (ARR) in primary aldosteronism (PA) screening have not been determined in a Korean population. Our study of 127 patients who underwent diagnostic testing for PA showed that the LC-MS/MS and RIA methods have good correlation, with a mean bias of 29.3% for PAC. An LC-MS/MS PAC level of 11.7 ng/dL was equivalent to an RIA PAC level of 15 ng/dL. Receiver operating characteristic curve analysis showed that an LC-MS/MS PAC level of 10.3 ng/dL and LC-MS/MS ARR level of 20.0 provided sensitivity of 73.1% with a specificity of 57.3% and sensitivity of 92.3% with a specificity of 14.7%, respectively. When the LC-MS/MS method is used for PA screening, an adjustment of cutoff values is necessary.

Keywords: Hyperaldosteronism; Aldosterone; Diagnosis; Chromatography, liquid; Tandem mass spectrometry; Radioimmunoassay

INTRODUCTION

Primary aldosteronism (PA) is the foremost endocrine cause of secondary hypertension, which is associated with a higher risk of target organ complications compared to essential hypertension (ET) [1,2]. A retrospective study of Korean patients showed that approximately 30% of hypertensive patients had positive screening results for PA, with 6% eventually confirmed for the disease, which suggests that PA may be more prevalent than generally recognized [3]. In this context, an accurate and sensitive screening test for PA is needed.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) for measuring plasma aldosterone concentration (PAC) and plasma renin activity (PRA) is highly accurate and reliable in diagnosing PA [4,5]. However, in the Korean population, immunoassay methods are predominantly used for measuring PAC and PRA, and the established cutoff values for PA are based on immunoassay measurements [6]. Moreover, comparative stud-

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ies between LC-MS/MS and immunoassay methods are scarce in the Korean population.

Our study aimed to evaluate the correlation between LC-MS/MS and radioimmunoassay (RIA), to determine the RIA equivalent value of LC-MS/MS, and to analyze optimal PAC cutoff values using LC-MS/MS for PA screening in a Korean population.

METHODS

Our study entailed a retrospective analysis of patients who were screened and underwent confirmatory testing for PA at Samsung Medical Center from January 2018 to November 2021. We reviewed 253 patients with hypertension (defined as systolic blood pressure [SBP] ≥140 mm Hg, diastolic blood pressure [DBP] ≥90 mm Hg, or use of antihypertensive medication) who underwent PAC and PRA assessment using both RIA and LC-MS/MS on the same day at an outpatient clinic. Inclusion was based on the presence of at least one of the following: younger than 40 years, grade 3 hypertension (SBP ≥160 mm Hg or DBP ≥100 mm Hg), hypertension with three or more antihypertensive medications, hypokalemia, adrenal mass, atrial fibrillation, a family history of PA in first-degree relatives, or a family history of stroke before the age of 40 [6]. The 82 individuals who did not meet these criteria were excluded. Thirty patients who had taken beta blockers, diuretics, or angiotensin receptor blockers within the prior 2 weeks or mineralocorticoid receptor antagonists in the prior 4 weeks to screening were excluded. We also excluded 14 patients who were suspected of autonomous cortisol secretion, defined as a cortisol level of more than 1.8 μg/dL after a 1 mg dexamethasone suppression test, or pheochromocytoma based on biochemical tests or imaging [7]. Consequently, 127 individuals were entered in the analysis.

We defined patients with PA or ET by the seated saline loading test using an LC-MS/MS PAC cutoff value of 5.8 ng/dL and RIA PAC cutoff value of 6.0 ng/dL [6,8]. Detailed explanations of the LC-MS/MS and RIA analytical methodologies are available in our preceding research paper [9]. Given that diabetes mellitus (DM) can influence PAC [10], we identified 31 patients with DM based on the prescription of DM medication, hemoglobin A1c ≥6.5%, or fasting blood glucose ≥126 mg/dL, to account for its potential impact on PAC measurements. An independent *t* test and the chi-square test were used to compare data between the PA and ET groups, and DM and non-DM groups. Passing-Bablok regression analysis and Bland-Altman analysis assessed the correlation and agreement between PAC and PRA by LC-MS/MS and RIA. The diagnostic performances and optimal cutoff values of PAC and aldosterone-to-renin ratio (ARR) by LC-MS/MS were evaluated using the receiver operating characteristic (ROC) curves analysis. Analyses were performed using MedCalc version 20 (MedCalc Software, Ostend, Belgium). The study protocol was approved by the Institutional Review Board (IRB) of Samsung Medical Center. The need for informed consent was waived (IRB No. 2023-02-053) because the study was retrospective and analyzed de-identified data.

RESULTS

Supplemental Table S1 shows the characteristics and screening test results for PA in both the PA-confirmed group and the ET group. In both groups, the levels of LC-MS/MS PAC and LC-MS/MS ARR were lower than those of RIA PAC and RIA ARR. PRA demonstrated no significant differences between the RIA and LC-MS/MS methods, which may be attributed to the general suppression of PRA in this study population, likely due to selection of patients with sufficient suspicion of PA to warrant confirmatory testing. T-tests shows no significant difference in LC-MS/MS PAC and RIA PAC between patients with and without DM (P=0.90 and P=0.76, respectively) (Supplemental Table S2).

According to Passing-Bablok regression analysis, the correlation between LC-MS/MS and RIA methods for PAC and PRA was good, with coefficients of 0.82 (95% confidence interval [CI], 0.76 to 0.87; P<0.001) (Fig. 1A) and 0.84 (95% CI, 0.77 to 0.88; P<0.001) (Fig. 1B), respectively. The regression equation was $y=-0.51+0.81\chi$ for PAC and $y=0.06+0.78\chi$ for PRA when χ was RIA and y was LC-MS/MS. According to the equation, an RIA PAC value of 15 ng/dL, the conventional threshold value for screening of PA, was equivalent to an LC-MS/MS PAC value of 11.7 ng/dL. Bland-Altman analysis showed that LC-MS/MS and RIA have a mean bias of 29.3% (95% CI, 24.0 to 34.5) (Fig. 1C) for PAC and a mean bias of 13.4% (95% CI, 5.9 to 20.9) (Fig. 1D) for PRA.

ROC curve analyses assessed the ability of LC-MS/MS PAC and LC-MS/MS ARR to discriminate between ET and PA patients (Supplemental Fig. S1). For the LC-MS/MS PAC and ARR, the area under the curve (AUC) values were 0.74 (95% CI, 0.66 to 0.81; P<0.001) and 0.70 (95% CI, 0.61 to 0.77; P<0.001), respectively. The AUC for RIA PAC was 0.78 (95% CI, 0.68 to 0.88), and for RIA ARR, it was 0.68 (95% CI, 0.58 to 0.78). Comparison between RIA and LC-MS/MS methods for both PAC and ARR yielded P=0.09 and P=0.98, respec-

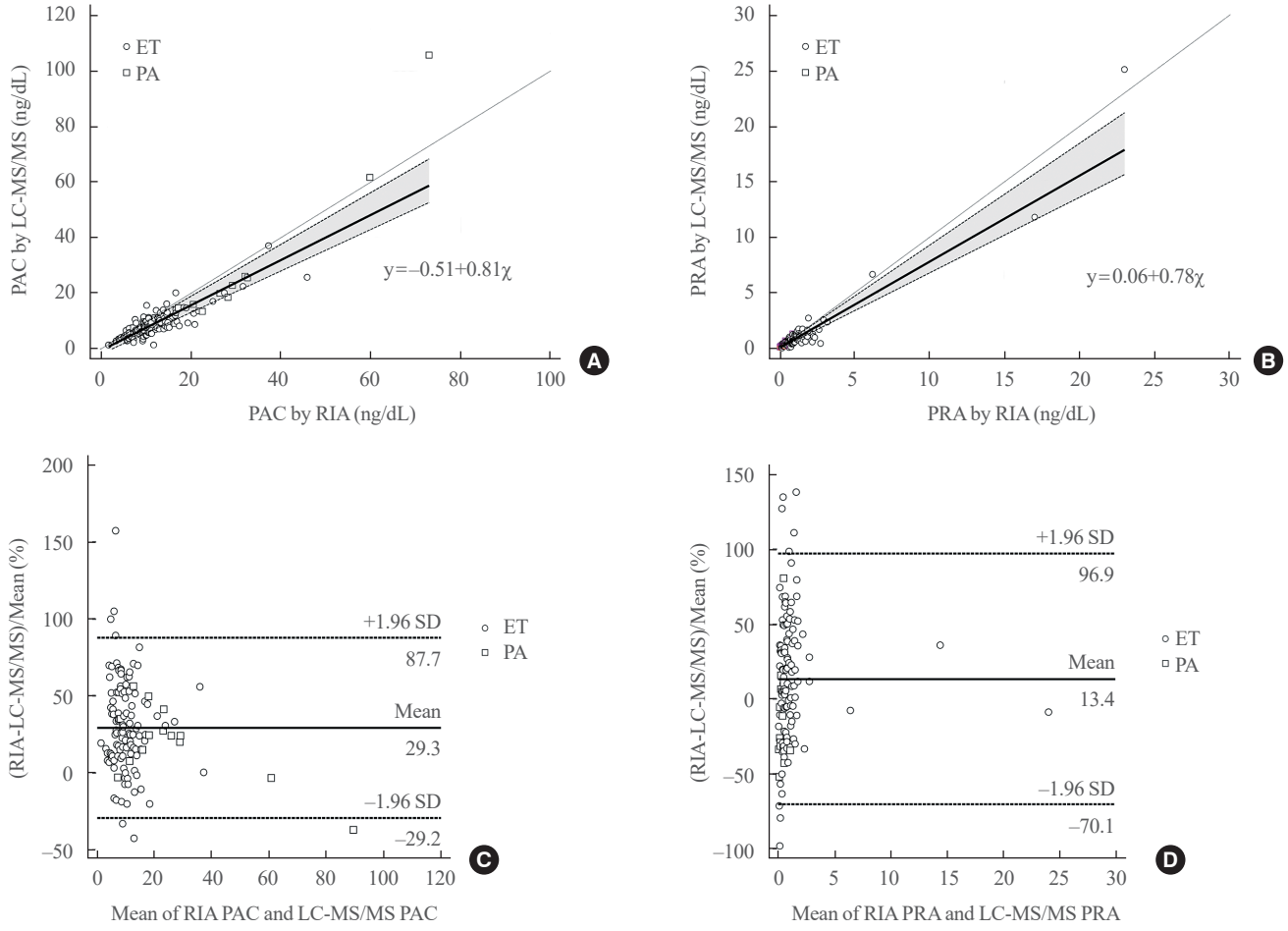

Fig. 1. Comparison of radioimmunoassay (RIA) and liquid chromatography-tandem mass spectrometry measurements of plasma aldosterone concentration (PAC) and plasma renin activity (PRA): Passing-Bablok regression analyses of (A) PAC and (B) PRA and Bland-Altman analyses of (C) PAC and (D) PRA. Essential hypertension (ET) is defined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) PAC as seated saline loading test ≤5.8 ng/dL; primary aldosteronism (PA) is defined by LC-MS/MS PAC as seated saline loading test >5.8 ng/dL (162 pmol/L). SD, standard deviation.

tively, indicating no significant differences. An LC-MS/MS PAC cutoff level of 10.3 ng/dL yielded a sensitivity of 73.1% and a specificity of 57.3%. An LC-MS/MS ARR cutoff level of 20.0 ng/dL per ng/mL/hr demonstrated a sensitivity of 92.3% and a specificity of 14.7% (Table 1). The discriminative ability of LC-MS/MS PAC and ARR to distinguish between PA and ET patients remains consistent when DM is included as a covariate (LC-MS/MS PAC AUC=0.74 [95% CI, 0.60 to 0.79]; LC-MS/MS ARR AUC=0.71 [95% CI, 0.62 to 0.76]) (Supplementary Fig. S2).

DISCUSSION

In this study, we evaluated the correlation of LC-MS/MS with RIA and the optimal cutoff values of LC-MS/MS PAC and ARR for screening PA in a Korean population with hypertension. Our results indicate a strong correlation and agreement between LC-MS/MS and RIA methods for measuring PAC and PRA. ROC curve analyses further supported the diagnostic utility of LC-MS/MS, with optimal cutoff levels for PAC and ARR (10.3 ng/dL and 20.0 ng/dL per ng/mL/hr, respectively) demonstrating reasonable sensitivity and specificity.

Our data indicated that the average PAC values obtained through LC-MS/MS were lower than those measured by RIA. This finding is consistent with the results of previous studies [9,11,12]. Guo et al. [11] demonstrated that RIA measurements are approximately 59% higher on average compared to LC-MS/MS. Similarly, Thuzar et al. [12] found RIA PAC were 17% to 29% higher than those obtained by LC-MS/MS. In our study, a mean bias of 29.3% was detected, which is within the range re-

Table 1. Performance of LC-MS/MS PAC and ARR Cutoff Values for Primary Aldosteronism Screening: Primary Aldosteronism vs. Essential Hypertension Confirmed by Seated Saline Loading Test

LC-MS/MS PAC, ng/dL	Sensitivity, %	Specificity, %	LC-MS/MS ARR, ng/dL per ng/mL/hr	Sensitivity, %	Specificity, %
>4.0	100.0	10.7	>12.0	100.0	5.3
>6.7	90.4	36.0	>15.0	96.2	6.7
>8.0	86.5	46.7	>20.0	92.3	14.7
>10.3	73.1	57.3	>25.6	86.5	17.3
>12.2	61.5	65.3	>31.0	82.7	20.0
>14.7	57.7	76.0	>39.0	80.8	33.3
>16.0	51.9	82.7	>50.0	76.9	50.7
>18.0	50.0	89.3	>61.0	69.2	61.3
>20.0	38.5	92.0	>70.0	61.5	72.0
>22.3	38.5	94.7	>80.0	57.7	74.7
>24.2	36.5	97.3	>100.0	44.2	84.0
>27.0	30.8	100.0	>200.0	23.1	100.0

LC-MS/MS, liquid chromatography-tandem mass spectrometry; PAC, plasma aldosterone concentration; ARR, aldosterone-to-renin ratio.

ported by these studies [11]. The lower PAC values in LC-MS/MS compared to RIA may stem from the cross-reactivity of the test method with steroids structurally similar to aldosterone [13]. Therefore, PAC values measured by immunoassays may exhibit reduced specificity relative to LC-MS/MS. This finding holds particular significance since our analysis revealed that the conventional RIA PAC threshold for PA screening (15 ng/dL) is equivalent to a lower LC-MS/MS value (11.7 ng/dL), and the optimal cutoff levels for LC-MS/MS PAC and LC-MS/MS ARR were lower than the conventional cutoff levels with immunoassay. This discrepancy underscores the need for revised cutoff values for LC-MS/MS to increase the accuracy of PA screening in clinical settings.

A notable limitation of this study is its single-center design, conducted in a tertiary hospital setting that predominantly included patients referred from primary care clinics. This could limit the generalizability of our findings. Therefore, conducting multi-center studies with varied population is essential for broader validation. While we established cutoff values for LC-MS/MS PAC and ARR in the Korean demographic, further research comparing other diagnostic methods is required to evaluate their relative effectiveness. Although the specificity of LC-MS/MS ARR in this study is low (14.7%), as noted in a previous meta-analysis, the specificity of ARR may be variable, and a single ARR cutoff cannot be recommended [14]. However, the high sensitivity of LC-MS/MS ARR (92.3%) in this study suggests its usefulness for screening PA patients who require further diagnostic testing. It is our hope that future research and revisions to clinical guidelines will consider these findings to enhance the precision of PA diagnosis when using LC-MS/MS.

In conclusion, adjusting cutoff values for PAC is necessary when the LC-MS/MS method is utilized for screening PA in clinical practice.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: S.Y.K., J.H.K. Acquisition, analysis, or interpretation of data: S.Y.K., K.J.K., S.Y.L. Drafting the work or revising: S.Y.K., J.H.K. Final approval of the manuscript: J.H.K.

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