Original Research Article

Development and validation of a novel chemiluminescent immunoassay for diagnosing primary aldosteronism

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Abstract

Purpose: To compare the diagnostic accuracy of plasma aldosterone concentration (PAC), plasma renin activity (PRA) and aldosterone-to-renin ratio (ARR) in primary aldosteronism (PA) using radioimmunoassay (RIA) and chemiluminescence immunoassay (CLIA) methods.

Methods: Both RIA and CLIA were used to analyze the PAC, PRA and ARR with subjects in standing or supine position, before and after a saline infusion test (SIT). The correlation between RIA and CLIA was measured by regression analysis. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic accuracy by RIA and CLIA.

Results: A positive correlation was found between PAC and PRA after SIT using RIA and CLIA (0.1745 and 0.3085, respectively). A positive correlation was found between the PAC and PRA in standing and supine position using RIA and CLIA (0.3979 vs 0.2399 and 0.1885 vs 0.4032, respectively). There was no obvious difference in AUCs of PAC, PRA, and ARR between RIA and CLIA (PAC: 0.91 vs. 0.89; PRA: 0.88 vs. 0.87; ARR: 0.93 vs. 0.92). In standing posture, the AUCs of PAC, PRA and ARR using RIA were 0.63, 0.72 and 0.78, respectively, and the results of CLIA were 0.65, 0.75 and 0.82, respectively. In supine posture, the AUC of PAC, PRA and ARR using RIA was 0.65, 0.68 and 0.71, respectively, and the results of CLIA were 0.68, 0.70 and 0.79, respectively.

Conclusion: Chemiluminescent assay is reliable for diagnosis of PA when compared with radioimmunoassay.

Keywords: Primary aldosteronism, Chemiluminescence immunoassay, Radioimmunoassay, Aldosterone Plasma renin activity, Aldosterone-to-renin ratio, Receiver operating characteristic

INTRODUCTION

Primary aldosteronism (PA) is a syndrome caused by either an adrenal mass or bilateral hyperplasia of the adrenals. The clinical manifestations of PA were inappropriate aldosterone hypersecretion and a low plasma level of renin [1]. As the main cause of secondary hypertension, more patients with PA experience cardiovascular and cerebrovascular disease when compared with essential hypertension patients [2,3]. Therefore, it is urgent to develop early and accurate diagnostic methods for PA.

Currently, many hospitals use ARR for diagnosing PA [4]. When the level of ARR exceeds the threshold, the patients need to be confirmed using suppression tests which including saline infusion suppression test (SIT), captopril challenge test, fludrocortisone suppression test and oral sodium loading test [5].

The Endocrine Society Clinical Practice Guidelines have suggested that a plasma aldosterone concentration (PAC) < 5 ng/dL after SIT is used to rule out PA, whereas PAC >10 ng/dL is an indicator for PA by using RIA [5,6]. However, it was found that PAC was always >5 ng/dl after SIT in clinical experience, it is this cutoff point was still controversial [7,8]. The RIA test is not only time-consuming, but also dangerous for workers because of its radioactive materials [9]. Hence, it is necessary to develop another effective method for screening PA. In 2004, Perschel reported a new method i.e., chemiluminescence immunoassay (CLIA), which was not only as effective as RIA but also solved the problems in RIA [10]. While the method of CLIA was still controversial, its diagnostic value in clinical practice has been reported to be promising [11].

It has been reported that different postures can affect the levels of renin and aldosterone [12]. Therefore, the different conditions of diurnal rhythm and posture are speculated as confounding factors for blood sampling. Due to the lack of postural standardization for PAC and PRA measurements, the posture used for screening PA in hypertensive patients is very different from the posture used in previous investigations.

The present study aimed to compare the diagnostic accuracy of PAC, PRA and ARR using CLIA and RIA methods. The diagnostic efficiency of PAC, PRA and ARR under different postures were also analyzed to identify the optimal cutoff value using CLIA.

EXPERIMENTAL

Patient selection

From March 2015 to August 2017, 114 PA patients, including 63 males and 51 females (aged from 23 to 73 years), were recruited from the Fourth Affiliated Hospital of Xinjiang Medical University, Urumqi, China. The patients were fully informed of the risk of participating in the trial, and they agreed to discontinue taking drugs that affected aldosterone and renin levels for more than two weeks before the research. The low blood potassium patients were adjusted to

normal level after hospitalization. Patients with cardiac and renal disorders were excluded. All patients signed informed consent forms. The study was approved by ethical approval committee of the Fourth Affiliated Hospital of Xinjiang Medical University (approval no. 2019XMU0113), and was conducted in accordance with the guidelines of Declaration of Helsinki [13].

Sample collection

The patients slept overnight in the hospital. At 7 am the next morning, 5 ml of cubital venous blood was collected when the patients were in clinostatism. Then, 5 – 15 min after sitting upright or walking for 1 h, 5 mL of cubital venous blood was collected to determine the plasma aldosterone and renin levels. The patients abstained from drinking water or consuming food during the period.

Saline infusion test

The patients were treated with 2 L of 0.9 % NaCl solution through intravenous infusion for about 4 hours (8 to 12 am) after overnight recumbency. Each patient was kept in a standing or supine position until the blood samples were collected for PRA and PAC before and after the infusion [6,14]. According to the guideline of Endocrine Society Clinical Practice, when the level of post-infusion plasma aldosterone is <5 ng/dL (140 pmol/L), the patients are not diagnosed as PA. Only when the level of post-infusion plasma aldosterone is >10 ng/dL (280 nmol/L), the patients are diagnosed as PA. Although the cutoff of 6.8 ng/dL (190 pmol/L) can guarantee the trade-off between sensitivity and specificity, the results of diagnosis are indeterminate when the level of post-infusion plasma aldosterone is between 5 ng/dL and 10 ng/dL [5]. Based on this criterion, 26 patients were diagnosed with PA, while 54 were non-PA cases.

Radioimmunoassay (RIA)

The RIA test was performed based on the guidelines reported in a previous study using a commercial kit (Jiuding Biological Technology LTD, Tian Jin, China) [15]. Briefly, the blood samples were divided into two aliquots (one kept at 37 °C and the other at 4 °C) to assayed the angiotensin I. The value of angiotensin I measured at 4 °C subtracted that determined at 37 °C was the PRA. RIA detection PAC was based on the principle of homogeneous competition. The radioactive iodine labeling was used to detect the aldosterone content in samples [16].

Chemiluminescence immunoassay (CLIA)

The fully automated chemiluminescence analyzer (Nichols Advantage®; Nichols Institute Diagnostics) was used to measure the levels of PAC. The solid phase of the system includes a sensitive acridinium ester detection technology and magnetic particles. Firstly, the samples, reagents, and magnetic particles were added into the disposable cuvettes to blend well. Then, the mixture were incubated at 37 °C for reaction and stopped by washing buffer, and the emitted light was calculated in relative light units (RLU). The stored master curve was considered as the standard cure for calibrating the system via a two-point recalibration method. The Nichols Advantage DirectRenin™ assay was used to measure the levels of PRA. There were two sites in the immunometric assay, and the acridinium-ester-labeled monoclonal antibody, a second biotinylated monoclonal antibody and streptavidin-coated magnetic particles were necessary for the assay. The incubation time was limited to 30 min at 37 °C to avoid the prorenin activation. This assay was calibrated according to World Health Organization reference material (National Institute for Biological Standards and Control code 68/356) [10].

Statistical analysis

The SPSS 22.0 software (IBM, USA) was used for statistical analysis. The results are presented as mean $\pm$ standard deviation (SD). For group comparisons of continuous variables, a two-tailed Student's *t*-test was used. The value of $p < 0.05$ was considered as statistically significant. The PAC, PRA, and ARR were presented as median and quartile spacing [M (P25, P75)]. Pearson's correlation analysis was used to analyze the correlation among PAC, PRA, and ARR using RIA and CLIA. The Wilcoxon rank sum test was used for analysis the between-the-groups comparison. Receiver operating characteristics (ROC) curves were used to determine the cutoff points that represent the maximum sensitivity and specificity of ARR.

RESULTS

Baseline characteristics of patients

Twenty-six patients with PA and 54 non-PA cases were recruited for this study. The demographics of the subjects are presented in Table 1. There were no significant differences in age, gender, systolic blood pressure (SBP), diastolic blood pressure (DBP), and serum potassium between PA and non-PA patients.

Effectiveness of using RIA and CLIA to determine PAC and PRA

To investigate the effect of CLIA, the PAC and PRA were determined, and the PAC/PRA ratio (**ARR**) was calculated using RIA and CLIA. As shown in Figure 1 A, a positive correlation was found between the PAC and PRA before and after SIT using RIA and CLIA (before SIT: PAC by RIA vs. PAC by CLIA ($R^2 = 0.1316$, *p < 0.0001*; PRA by RIA vs. PRA by CLIA ($R^2 = 0.4156$, *p < 0.0001*); after SIT: PAC by RIA vs. PAC by CLIA, ($R^2 = 0.1745$, *p < 0.0001*); PRA by RIA vs. PRA by CLIA ($R^2 = 0.3085$, *p < 0.0001*). These results suggested that there was no significant difference between RIA and CLIA to detect PAC and PRA. To determine the difference between the PAC and PRA when a subject was standing or in a supine position, the PAC and PRA in supine or standing position were detected by using RIA and CLIA. As shown in Figure 1 B, in the supine position, the PAC value was calculated by RIA and CLIA had a positive correlation ($R^2 = 0.1885$, $p < 0.001$), and the PRA value detected by RIA and CLIA also had a strong positive correlation ($R^2 = 0.4032$, $p < 0.001$). When standing, the PAC value was calculated by RIA and CLIA had a positive correlation ($R^2 = 0.3979$, $p < 0.001$), and the PRA value detected by RIA and CLIA also had a strong positive correlation ($R^2 = 0.2399$, $p < 0.001$).

Table 1: Demographic characteristics of subjects

Characteristic	PA	Non-PA	*P-vakue*
Age (years)	49.73±9.59	45.22±9.64	0.901
Sex (Male/Female)	6/20	34/20	0.214
SBP (mmHg)	142.23±15.32	140.20±15.26	0.897
DBP (mmHg)	91.54±11.42	85.73±9.72	0.653
s-K+ (mmol/L)	3.50±0.41	3.84±0.31	0.026

s-K+= serum potassium; SBP= systolic blood pressure; DBP= diastolic blood pressure; PA= primary aldosteronism
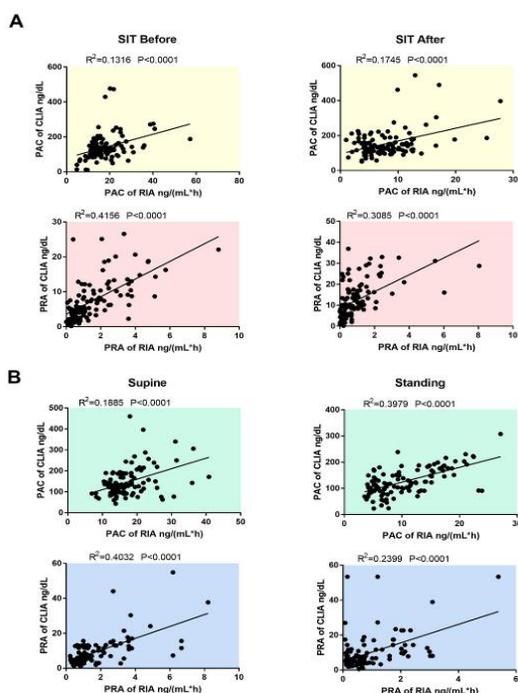
Figure 1: Correlation of PAC and PRA measurements between RIA and CLIA. (A) Scatter plots showing the ratio of PAC/PRA ratio measured with RIA and CLIA before and after SIT. (B) Scatter plots showing the ratio of PAC/PRA ratio measured with RIA and CLIA in a supine and standing posture

Diagnostic accuracy of PAC, PRA and ARR using RIA and CLIA

The cutoff point value with the two different methods after SIT was also compared. As shown in Table 2, the cutoff points of PAC, PRA and ARR using RIA was > 6.8 ng/dL, <1 ng/ml/h and > 6.8, respectively. As shown in Table 3, the cutoff point of PAC, PRA and ARR using CLIA was >115.09 pg/mL, ≤ 4.51 pg/mL and >25.52, respectively. Moreover, the sensitivity, specificity and the area under the curve (AUC) between RIA and CLIA were also compared. There were no obvious difference in the AUC of PAC, PRA,

and ARR between RIA and CLIA (PAC: 0.91 vs. 0.89; PRA: 0.88 vs. 0.87; ARR: 0.93 vs. 0.92, Figure 2). These results indicated that CLIA was as effective as RIA in detecting PA.
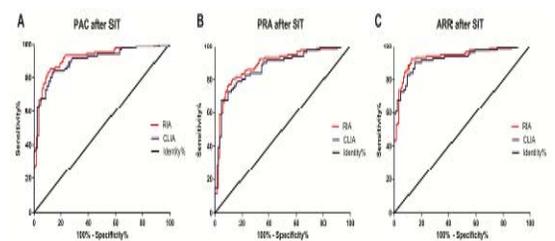
Figure 2: ROC curves for PAC (A), PRA (B) and ARR (C) after SIT by using RIA and CLIA

The ROC curves of PAC, PRA, and ARR values under different posture conditions using RIA were presented in Table 4. The cutoff point of PAC, PRA and ARR by using RIA in a standing posture were >18.4 ng/dL, <1.3 ng/ml/h and > 14.15, respectively. The AUC of PAC, PRA and ARR using RIA in a standing posture was 0.85, 0.87 and 0.90, respectively (Figure 3 A - C). In addition, the cutoff point of PAC, PRA and ARR using RIA in a supine posture were >7.90 ng/dL, <0.60 ng/ml/h and > 13.17, respectively. The AUCs of PAC, PRA and ARR were respectively 0.86, 0.87 and 0.92 in a supine posture by using RIA (Figures 3 D - F). The Table 5 had showed the ROC curves of PAC, PRA, and ARR under the two posture conditions by CLIA. The CLIA was used to analyze the cutoff point of PAC, PRA and ARR in a standing posture, and the results were >225.79 pg/mL, ≤ 6.38 pg/mL and >27.27, respectively. The AUCs of PAC, PRA and ARR using CLIA in a standing posture was 0.82, 0.85 and 0.88, respectively (Figures 3 A - C). Moreover, the cutoff point of PAC, PRA and ARR using CLIA in a supine posture was >120.95 pg/mL, ≤ 4.59 pg/mL and >38.36, respectively.

Table 2: Performance of PAC, PRA and ARR after SIT by using RIA

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	95% CI	*P*-value
PAC (ng/dL)	> 6.8	87.72	98.25	0.91	0.87-0.95	
PRA (ng/mL/h)	< 1	85.34	94.28	0.88	0.84-0.93	<0.0001
ARR	> 6.8	89.54	99.21	0.93	0.89-0.97	<0.0001

Table 3: Performance of PAC, PRA and ARR after SIT by using CLIA

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	95% CI	*P*-value
PAC (pg/mL)	> 115.09	85.48	92.31	0.89	0.85-0.95	<0.0001
PRA (pg/mL)	≤ 4.51	82.53	88.65	0.87	0.81-0.92	<0.0001
ARR	> 25.52	89.48	94.58	0.92	0.88-0.96	<0.0001

The AUC of PAC, PRA and ARR using CLIA in a supine posture were 0.84, 0.86 and 0.89, respectively (Figure 3 D and F). The results showed that there was no significant differences in the diagnostic accuracy of PAC, PRA and ARR by RIA and CLIA.
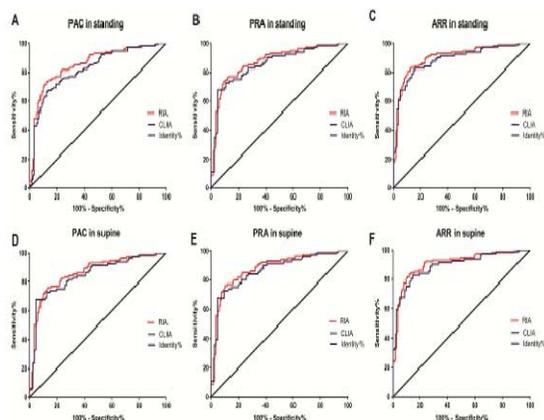
Figure 3: ROC curves for PAC, PRA and ARR between different positions by using RIA and CLIA. The ROC curves for PAC (A), PRA (B) and ARR (C) in standing position using RIA and CLIA. The ROC curves for PAC (D), PRA (E) and ARR (F) in supine position by using RIA and CLIA

DISCUSSION

Due to an adrenal disorder, PA is characteristic with overproduction of aldosterone to induce the endocrine hypertension, which is about 10 % of all hypertensive patients [17]. The guidance of ES for PA diagnosis includes screening, confirmatory testing and subtype differentiation [14]. Patients with PA exhibit a high risk of cardiovascular and cerebrovascular conditions with essential hypertension. However, patients with PA are successfully treated for hypertension with appropriate interventions such as surgery or treatment with a mineralocorticoid receptor antagonists [6,18]. These anti-hypertensive medications and testing conditions may affect the accuracy of ARR [14]. The early diagnosis for hypertensive patients with of PA is useful for clinical treatment.

The ARR is calculated from the ratio of PAC to PRA, which is used as the most common method for PA screening test. Although RIA is considered the best validated screening protocol for PA after SIT, there are still many drawbacks in RIA. The RIA for measuring PRA requires cooling of the specimen during transport and storage. The RIA is a time-consuming method, which has poor inter-laboratory reproducibility. The PRA, PAC, and ARR show large intra- and inter-patient variations [19]. Previous studies have reported on the development an alternative screening procedure that could overcome the above-mentioned disadvantages [9,10,20,21]. The sensitivity and specificity of diagnosis showed an excellent result through gas-chromatography or liquid chromatography with mass spectrometry.

Table 4: Performance of PAC, PRA and ARR between different positions by using RIA

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	95% CI	*P*-value
Standing						
PAC (ng/dL)	> 18.40	81.22	85.33	0.85	0.80-0.90	<0.0001
PRA (ng/ml/h)	< 1.30	84.27	87.26	0.87	0.82-0.91	<0.0001
ARR	> 14.15	88.27	91.52	0.90	0.86-0.94	<0.0001
Supine						
PAC (ng/dL)	> 7.90	82.81	86.58	0.86	0.81-0.91	<0.0001
PRA (ng/ml/h)	< 0.60	84.12	88.32	0.87	0.83-0.92	<0.0001
ARR	> 13.17	87.54	92.68	0.92	0.86-0.94	<0.0001

Table 5: Performance of PAC, PRA and ARR between different positions by using CLIA

Variable	Cut-off point	Sensitivity (%)	Specificity (%)	AUC	95% CI	*P* value
Standing						
PAC (pg/mL)	> 225.79	79.58	82.37	0.82	0.76-0.87	<0.0001
PRA (pg/mL)	≤ 6.38	81.53	84.28	0.85	0.79-0.91	<0.0001
ARR	> 27.27	84.52	88.64	0.88	0.83-0.93	<0.0001
Supine						
PAC (pg/mL)	> 120.95	81.54	85.72	0.84	0.78-0.90	<0.0001
PRA (pg/mL)	≤ 4.59	84.52	87.59	0.86	0.80-0.92	<0.0001
ARR	> 38.36	85.48	89.56	0.89	0.84-0.94	<0.0001

However, the sample preparation for mass spectrometry is also difficult and time consuming [22]. Due to these drawbacks, these methods cannot be widely used in clinical tests.

In the present study, the CILA and RIA were used to test the PAC, PRA, and ARR through SIT. The regression curves indicated that there was a positive correlation between the PAC and PRA before and after SIT using RIA and CLIA. Moreover, all the AUC of PAC, PRA, and ARR exceeded 0.7 after SIT using RIA and CLIA. There were no obvious difference in the AUC of PAC, PRA, and ARR after SIT between RIA and CLIA. These results indicated that CLIA was as effective as RIA in testing PAC PAC, PRA, and ARR after SIT.

It is well accepted that circadian variation and posture are two important and interacting factors for PAC, PAR, and ARR. Previous studies have reported that PRA increased significantly at the 5th minute, and, after 120 min of upright posture, its value was three-times greater than its value after the patient was in the supine position. They also demonstrated that the levels of renin and aldosterone significantly increased after remaining upright for 10 min in comparison to the baseline levels [23]. Moreover, ARR was measured while maintaining a standing posture in hypertensive patients, whereas the ARR measured while maintaining a standing posture for two hours was lower in patients with essential hypertension. Therefore, blood samples taken at different times and under different posture conditions will be affected by the amount of time spent maintaining the posture before obtaining the blood sample.

In this study, the PAC, PRA, and ARR under different posture conditions were further tested by using RIA and CLIA. A positive correlation was found between the PAC and PRA both in the standing and supine positions using RIA and CLIA (Figure 1 B). There was also no significantly difference in the diagnostic accuracy of PAC, PRA and ARR by RIA and CLIA in the standing and supine posture.

CONCLUSION

The chemiluminescence method used in this study is a robust and comparable technique for case detection and confirmation of PA when compared with the classical radioimmunometric method. This chemiluminescence immunoassay is also an automated, reliable and non-radioactive, which progressively promoted widespread use of the aldosterone and renin measurement in the future.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Authors' contributions

Hong-Feng Xu and Li Ge designed research. Li Ge and Jian-Jun Ma performed research. Jian-Jun Ma, Meng-Ru Wu, Yun Jia and Ya-Li Xu contributed samples or analytic tools. Hong-Feng Xu and Li Ge analyzed data and wrote the paper. Hong-Feng Xu supervised the experiments, revised and approved the manuscript.

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