CORRELATION BETWEEN PLASMA RENIN CONCENTRATION AND PLASMA RENIN ACTIVITY ASSAYS ON THE MAGLUMI 800 CHEMILUMINESCENCE IMMUNOASSAY ANALYZER

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The ratio of plasma aldosterone concentration to plasma renin activity (PAC/PRA) is the most common screening test for primary hyperaldosteronism, but it is not standardized among laboratories. We evaluated the correlation between plasma renin concentration (PRC) and plasma renin activity (PRA), and then PAC/PRC and PAC/PRA ratios on Maglumi 800 immunoassay analyzer. Forty-five plasma samples from volunteers were collected. PRC, PRA and PAC were measured by Maglumi 800 - a fully-auto chemiluminescence immunoassay analyzer. Correlation between plasma renin concentration (PRC) and plasma renin activity (PRA), and then PAC/PRC and PAC/PRA ratios were determined by regression analysis. PRC and PRA showed a good correlation (r = 0.88). The PAC/PRC and PAC/PRA ratios have a similar relationship (r = 0.757). Because of the advantages of the procedure and the independence of PRC from endogenous angiotensinogen levels, PRC may be used preferentially over PRA as high throughput screening tool.

Keywords: Plasma renin activity (PRA), plasma renin concentration (PRC), plasma aldosterone concentration to plasma renin activity ratio (PAC/PRC), plasma aldosterone concentration to plasma renin concentration ratio (PAC/PRA).

I. INTRODUCTION

The renin-angiotensin system (RAS) is best known for its important role in the physiological regulation of blood pressure through peripheral and central mechanisms. Currently, the RAS system is defined as a system consisting of different angiotensin peptides with diverse biological roles mediated by distinct receptor subtypes.[1] Due to the complex and multifunctional aspects of this system, as well as the growing concern for interlaboratory consistency, many biochemical methods have now been developed for the characterization and identification of these different components to reflect the status of RAS. Plasma renin activity (PRA), plasma aldosterone concentration (PAC) and PAC/PRA ratio are the tests used to diagnose and monitor diseases related to the activity of the renin - angiotensin - aldosterone system. Plasma renin activity (PRA) was determined by the amount of angiotensin I formed by renin catalysis from angiotensinogen per unit time. Although PRA is convenient for assessing the biological activity of the renin-angiotensin system, it may not reflect the true concentration of active renin.[2] Substrate concentrations rarely affect PRA results, however in some cases PRA results are limited by the body's physiological angiotensinogen levels.[3] More importantly, PRA depends not only on renin, but also on factors influencing renin-substrate (angiotensinogen) interaction with renin, related to the conditions of the enzymatic reaction.

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The plasma renin concentration (PRC) assay directly measures the concentration of active renin in the plasma, independent of the amount of angiotensinogen. This method has the advantage of being simple, fully automatic, and has a fast response time. Determining the correlation between PRC and PRA is the basis for using PRC and PAC/PRC ratio in assessing the activity of the renin - angiotensin - aldosterone system. The aim of this study was to determine the correlation between PRC and PRA, PAC to PRC and PAC to PRA ratios.

II. MATERIALS AND METHODS

1. Subjects

Plasma samples were obtained from forty-five volunteers aged from one month to 65 years old including 30 healthy, normotensive volunteers (15 males aged from 22 to 43 years old and 15 females aged from 24 to 60 years old), who were not taking any relevant medications and 15 patients aged from 1 month to 16 years old with symptoms of hypertension or water-electrolyte disturbance with different etiologies.

Sample collection and handling

Blood samples were drawn from patients in an upright body position or lying down position from a forearm vein between 8 am and 9 am. In direct renin assay and aldosterone assay, blood samples were collected in EDTA tubes, spined down in a non-refrigerated centrifuge for 5 min at 3500 round per min, plasma was separated from cells immediately after centrifugation, then aliquoted and deep-frozen at -20°C instantly. In Angiotensin I assay, 2 mL blood samples were collected in EDTA-2K tubes with10 µL Dimercaprol and 20 µL enzyme inhibitors added to each tube. After capping, the tubes were inverted up and down several times for mixing, and placed in 4°C refrigerator for 1-2 hours; the tubes were subsequently centrifuged at 4°C for 7 minutes at 4020 round per min to separate the plasma and then deep- frozen at (-20)°C instantly.

Research ethics

The study protocol was approved by the Ethics Committee for Biomedical Research, Vietnam National Hospital of Pediatrics (certificate no. 3183/BVNTW-HĐĐĐ) and informed consent was given by all participants.

2. Assays

Plasma aldoterone, direct renin and renin activity assays were measured on Maglumi 800 using Snibe test kits. The assay procedures were performed in accordance with the manufacturer's instructions.

The aldosterone assay is a competitive chemiluminescence immunoassay. Sample (calibrator/control, if applicable), ABEI labeled with anti-ALD monoclonal antibody, FITC labeled with purified ALD antigen, magnetic microbeads coated with sheep anti-FITC polyclonal antibody were mixed thoroughly and incubated at 37 °C, forming antibody-antigen complexes. After precipitation in a magnetic field, the supernatant was decanted, followed by a wash cycle. Subsequently, Starter 1+2 were added to initiate a chemiluminescent reaction. The light signal was measured by a photomultiplier within 3 seconds as relative light units (RLUs), which was inversely proportional to the concentration of aldosterone present in the test sample (or calibrator/control, if applicable).

The direct renin assay is a sandwich chemiluminescence immunoassay. The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-Renin monoclonal antibody, and ABEI labeled with another anti-Renin monoclonal antibody were mixed thoroughly and incubated at 37

°C, forming a sandwich immuno-complexes. After precipitation in a magnetic field, the supernatant was decanted followed by a wash cycle. Subsequently, Starter 1+2 were added to initiate a chemiluminescent reaction. The light signal was measured by a photomultiplier within 3 seconds as relative light units (RLUs), which was proportional to the concentration of Renin present in the sample (or calibrator/control, if applicable).

Plasma renin activity was measured by the determination of the amount of angiotensin I generated after incubation of plasma at 37°C. The Angiotensin I assay is a competitive chemiluminescence immunoassay. Preparation for analysis AI including three steps:

1. Plasma was divided into two parts, both parts were combined with pH Regulator by 1:8 ratio (100 µL pH Regulator + 800 µL plasma) to adjust the specimen pH value.

2. One part was kept in an ice-water bath (0 to 4 °C) directly. The other part was incubated in 37 °C water bath for 1 hour and then kept in ice-water bath (0 to 4 °C).

3. These two parts of sample were loaded simultaneously r to the analyzer and tested almost at the same time.

In Maglumi 800, the sample (or calibrator/control, if applicable), ABEI-labeled AI antigen, and magnetic microbeads coated with anti-AI polyclonal antibody were mixed thoroughly and incubated at 37 °C. Then the AI in the sample and ABEI Label competed for binding the magnetic microbeads, forming immuno-complexes.. The testing procedure was similar to the aldosterone assay. PRA is calculated from AI according to the formula:

Plasma rennin activity (PRA) = AI's concentration of sample in 37°C incubation - AI's concentration of samples in ice bath.

The ratio of PAC to PRA and PAC to PRC was calculated for all samples.

Method validation experiments according to CLSI EP 15-A3 were performed for all 3 assays. Calibration results were determined via a calibration curve which was instrument-specifically generated by 2-point calibration. Quality control analysis was included and samples were analyzed only when quality control results felt within acceptable ranges for each assay.

3. Statistical evaluation

Using SPSS 20, a Spearman's rank correlation analysis was used to determine the relationship between variables.

III. RESULTS

The study samples were obtained from 45 individuals, with different PRA values. As presented in table 1, samples from 1 to 15 were from patients. Samples from 16 to 45 were from healthy volunteers. The highest value of PRA is 194.14 (ng/mL/hour) and the lowest value is 0.103 (ng/mL/hour).

Table 1. Plasma renin activity (PRA), plasma renin concentration (PRC) and plasma aldosterone concentration (PAC) results as well as PAC to PRA and PAC to PRC ratios in study subjects

Sample	PRA (ng/mL/hour)	PRC (µIU/mL)	PAC (pg/mL)	PAC/PRC (pg/µIU)	PAC/PRA (pg/mL)/ (ng/mL/hour)
P1	3.298	49.8	140	2.81	42.45
P2	0.103	2.55	39.5	15.49	383.50

Sample	PRA (ng/mL/hour)	PRC (µIU/mL)	PAC (pg/mL)	PAC/PRC (pg/µIU)	PAC/PRA (pg/mL)/ (ng/mL/hour)
P3	99.7	867	1638	1.89	16.43
P4	1.752	19.3	61.3	3.18	34.99
P5	12	147	457	3.11	38.08
P6	2.1	307	39.1	0.13	18.62
P7	0.153	7.64	44.8	5.86	292.81
P8	4.59	61.6	340	5.52	74.07
P9	33.49	215	997	4.64	29.77
P10	18.51	217	172	0.79	9.29
P11	194.14	5020	1412	0.28	7.27
P12	3.02	52.7	136	2.58	45.03
P13	18.9	226	86	0.38	4.55
P14	2.15	43.6	348	7.98	161.86
P15	6.13	99.7	582	5.84	94.94
V16	1.41	19.20	122.00	6.35	86.28
V17	2.82	13.50	125.00	9.26	44.33
V18	3.97	44.40	168.00	3.78	42.32
V19	2.12	34.20	147.00	4.30	69.50
V20	1.78	25.40	128.00	5.04	71.91
V21	1.57	23.80	88.40	3.71	56.31
V22	1.66	27.20	150.00	5.51	90.36
V23	2.11	26.80	142.00	5.30	67.30
V24	4.95	61.90	481.00	7.77	97.17
V25	2.99	40.80	209.00	5.12	69.90
V26	1.22	19.30	124.00	6.42	101.64
V27	3.55	52.80	257.00	4.87	72.39
V28	1.99	24.30	134.00	5.51	67.34
V29	1.73	17.60	129.00	7.33	74.70
V30	1.47	17.20	128.00	7.44	87.25
V31	1.43	14.30	125.00	8.74	87.41
V32	0.59	8.44	212.00	25.12	357.50
V33	0.54	20.90	104.00	4.98	192.59
V34	3.47	50.90	202.00	3.97	58.21
V35	1.81	34.50	73.90	2.14	40.83
V36	1.94	49.80	133.00	2.67	68.56

Sample	PRA (ng/mL/hour)	PRC (µIU/mL)	PAC (pg/mL)	PAC/PRC (pg/µIU)	PAC/PRA (pg/mL)/ (ng/mL/hour)
V37	1.77	33.30	136.00	4.08	76.84
V38	1.22	14.20	103.00	7.25	84.22
V39	5.21	61.40	231.00	3.76	44.34
V40	1.45	19.20	133.00	6.93	91.72
V41	3.52	48.30	109.00	2.26	30.97
V42	0.21	5.99	26.80	4.47	127.62
V43	1.45	25.80	75.70	2.93	52.32
V44	2.32	44.90	115.00	2.56	49.57
V45	0.77	33.80	69.60	2.06	89.92

PRA is best used for measurement of low renin levels; both PRA and PRC are suitable for normal and high renin levels. Determination of PRC in contrast to PRA offers advantages with regard to processing and standardization. Therefore, determining the correlation between PRC and PRA is the basis for using PRC in assessing the activity of the renin - angiotensin - aldosterone system.

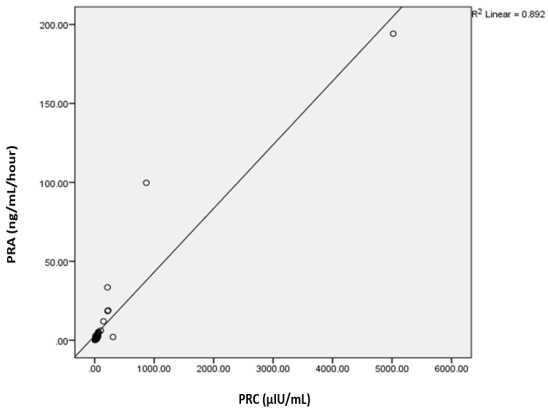

Figure 1. Correlation between plasma renin concentration (PRC) and plasma renin activity (PRA)

Spearman's test demonstrated a significant correlation for the PRA and PRC with r = 0.880 ($p < 0.01$). In healthy volunteers, Spearman's test demonstrated a significant correlation for the PRA and PRC with r = 0.767 ($p < 0.01$). In patients, Spearman's test demonstrated a significant correlation for the PRA and PRC with r = 0.825 ($p < 0.01$). There is a good linear correlation between PRA and PRC.

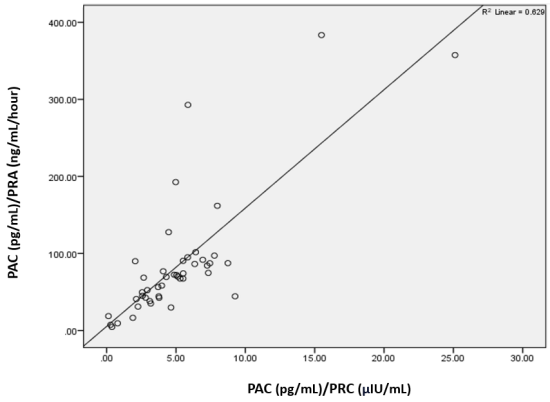

Figure 2. Correlation between plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratios and plasma aldosterone concentration (PAC) to plasma renin concentration (PRC) ratios

Spearman's test demonstrated a significant correlation for the PAC to PRA and PAC to PRC ratio with r = 0.757 ($p<0.01$). In healthy volunteers, Spearman's test demonstrated a significant correlation for the PAC to PRA and PAC to PRC ratio with r = 0.547 ($p=0.002<0.01$). In patients, Spearman's test demonstrated a significant correlation for the PAC to PRA and

PAC to PRC ratio with r = 0.886 (p<0.01). The PAC results in the healthy group are lower than the patient group, so when evaluating the correlation between the PAC to PRA and PAC to PRC ratio, the correlation is weaker than the patient group. However, p=0.002<0.01, r=0.547 still shows a close linear relationship. There is a good linear correlation between PAC/PRA ratio and PAC/PRC ratio.

IV. DISCUSSION

The renin - angiotensin - aldosterone system plays an important role in regulating and maintaining blood circulation and blood pressure. Disturbances of this system can be the cause of metabolic imbalances and diseases. Some disorders caused by abnormal functioning of the renin - angiotensin - aldosterone system such as: Conn's syndrome and other forms of primary mineralocorticoid excess, renal artery stenosis, Bartter's syndrome, Renin-secreting tumours, pseudohypoaldosteronism, primary aldosterone deficiency, secondary aldosterone deficiency, and assessment of the renin-angiotensin-aldosterone system during the investigation of these disorders was performed.[4] In addition, inactive prorenin has been suggested as a marker of disease progression in neuroblasts[5] and an early marker of microvascular complications in diabetes.[6,7] Two parameters frequently identified when evaluating the renin-angiotensin-aldosterone system are renin and aldosterone. In Conn's syndrome, or primary hyperaldosteronism, high circulating aldosterone levels are expected when renin levels are low, whereas in renal artery disease both aldosterone and renin will be elevated. Renin and aldosterone are also measured to evaluate patients with congenital adrenal hyperplasia, pseudohypoaldosteroism, and renin-secreting tumors. Addison's disease is characterized by underactive adrenal glands. However, depending on the cause of Addison's disease, aldosterone levels may not be affected. Aldosterone is affected only in Addison's disease when the adrenal gland is destroyed, for example, during autoimmune-mediated destruction. Aldosterone is controlled by the renin-angiotensin system, while the rest of the adrenal gland's hormone production is controlled by adrenocorticotropic hormone (ACTH). Therefore, cases of Addison's disease due to pituitary dysfunction will present with adrenal insufficiency but with appropriate aldosterone levels. This is because the renin-angiotensin system remains intact.[8]

The aldosterone/renin ratio (ARR) is the ratio of aldosterone concentration divided by renin activity or renin concentration. The aldosterone/renin ratio is recommended as a screening tool for primary hyperaldosteronism.[9] Primary hyperaldosteronism (PHA) is recognized as the most common endocrine form of secondary hypertension with an estimated prevalence of 5% to 15% in the hypertensive population [9]. Early detection of PHA facilitates effective therapy, so expanded screening to include patients with normal blood K^+ levels is widely accepted .[10,11,12] The most common PHA screening test is the ratio of plasma aldosterone concentration to plasma renin activity (PAC/PRA).[10,12] Because plasma renin activity (PRA) measurement requires rigorous reaction conditions, it is time consuming and has poor inter-laboratory consistency, this test is not recommended for the screening of hypertensive patients in primary health care centers .[13,14,15] Recently developed immunoassays for the quantification of plasma renin concentration levels (PRCs) can overcome these limitations. Determining the correlation between PRC and PRA is the basis for using PRC and PAC/PRC ratio in assessing the activity of the renin - angiotensin - aldosterone system. The aim of our study

was to determine the correlation between PRC and PRA, PAC to PRC and PAC to PRA ratios. Forty-five plasma samples from volunteers (30 healthy volunteers and 15 patients) were collected. PRC, PRA and PAC were measured by Maglumi 800 - a fully-auto chemiluminescence immunoassay analyzer. The obtained results show that PRC and PRA showed a good correlation (r = 0.88). The PAC/PRC and PAC/PRA ratios have a similar relationship (r = 0.757). Because of the advantages of the procedure and the independence of PRC from endogenous angiotensinogen levels, PRC may be used preferentially over as high throughput screening tool.

Several trials to determine the cut-off of the PAC/PRC ratio in PHA screening as well as to evaluate the correlation between the PAC/PRA ratio and the PAC/PRC ratio were performed. Our study has similar results with others Studies. Deirdre´ Hartman et al conducted a comparative study of direct renin quantification and measurement of renin activity.[2] The aim of this study was to determine the relationship between direct renin concentration (PRC) values and renin activity (PRA) in hypertensive patients, especially in patients with very low PRA values, with the goal of finding a fast and efficient screening method. The study sample was obtained from 111 individuals, of which 34 were on treatment for hypertension and 77 were not on treatment. Renin activity (PRA) was determined through quantification of angiotensin I levels by radioimmunoassay. Direct renin quantification (PRC) by chemiluminescence immunoassay. Linear regression was used to evaluate the correlation between PRA and PRC results. The correlation coefficient between PRA results at different levels and corresponding PRC results is 0.98 (n=110). The PRC test can measure low renin levels in samples with PRA <0.65 ng/mL/hour with good correlation and low CV. This suggests that the quantitative method of PRC can be used to evaluate renin activity, especially with samples with low renin concentration. The performance advantage of the quantitative PRC test makes it an effective tool in community studies and in screening for secondary forms of hypertension, including primary hyperaldosteronism and Liddle syndrome.

Frank Holger Perschel et al studied a rapid screening test for primary hyperaldosteronism.[16] The ratio of plasma aldosterone concentration to plasma renin activity (PAC/PRA) is a commonly used indicator in screening for primary hyperaldosteronism (PHA). However, there is no standardized method for measuring renin activity between laboratories. This study evaluates new automated tests for the simultaneous measurement of plasma aldosterone concentration (PAC) and plasma renin concentration (PRC). The study sample was obtained from 76 normal healthy volunteers and 28 patients with PHA. PAC and PRC were measured using an automated machine according to the principle of chemiluminescence. Analysis of the obtained data shows that the results of single measurements of PAC, PRA or PRC may show some similarity between the healthy population and the group of PHA patients. Meanwhile, the ratio of PAC/PRA and PAC/PRC has a clear difference between these two groups. PRC and PRA showed good correlation (r=0.72). Since the PACs are the same, the PAC/PRC and PAC/PRA ratios are similarly correlated (r=0.73). The PAC/PRC ratio offers several advantages over screening using the PAC/PRA ratio method. The present study provides preliminary evidence that the PAC/PRC ratio is a useful screening test for the detection of PHA.

Nicole Unger et al compared plasma renin concentration and plasma renin activity to diagnose primary hyperaldosteronism in patients with adrenal tumors.[17] The ratio of plasma aldosterone concentration (PAC) to plasma renin activity (PRA) is the test used to screen for primary hyperaldosteronism. Determination of plasma renin concentration (PRC) offers an advantage over measurement of renin activity (PRA) in terms of performance and standardization. Comparative study of PRA and PRC under randomized conditions to establish diagnostic thresholds for primary hyperaldosteronism. Fifty patients with different adrenal tumors, including 10 patients with aldosterone-secreting adenomas, 10 hypertensive patients, and 23 non-hypertensive volunteers participated in the study. PAC and PRA were measured by radioimmunoassay. PRC was determined by immunoassay. Analyzing the obtained results, the study proposed that the threshold for the PAC/PRC ratio is 90 ((ng/l)/(ng/l)) (sensitivity 100%, specificity 98.6%) and threshold ratio 62 by further considering a PAC ≥ 200 ng/l (100% sensitivity, 100% specificity) for the diagnosis of aldosterone-secreting adrenal adenoma. A PAC/PRC ratio of 62 in patients with a PAC level 200 ng/l is a reliable screening method for the detection of primary hyperaldosteronism in patients with aldosterone-secreting adrenal adenomas under randomized conditions.[17]

V. CONCLUSION

In summary, PRC and PRA showed a good correlation. The PAC/PRC and PAC/PRA ratios have a similar relationship. Because of the advantages of the procedure and the independence of PRC from endogenous angiotensinogen levels, our results suggest that PRC may be used preferentially over as high throughput screening tool.

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