Diagnostic accuracy of plasma free metanephrines in a seated position compared with 24-hour urinary metanephrines in the investigation of pheochromocytoma

Hye Jeong Kim1)*, Ji In Lee2)*, Yoon Young Cho3), Soo Youn Lee4), Jung Han Kim5), Byong Chang Jung6), Sun Wook Kim3), Jae Hoon Chung3), Yong-Ki Min3), Myung-Shik Lee3), Moon-Kyu Lee3) and Jae Hyeon Kim3)

1) Division of Endocrinology and Metabolism, Department of Internal Medicine, Soonchunhyang University Hospital, Soonchunhyang University College of Medicine, Seoul, Korea
2) Department of Internal Medicine, National Police Hospital, Seoul, Korea
3) Division of Endocrinology and Metabolism, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
4) Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
5) Division of Breast and Endocrine Surgery, Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea
6) Department of Urology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract. The aim of this study was to determine the diagnostic efficacy of free metanephrines in plasma samples drawn in the seated position compared with 24-h urinary metanephrines in detecting pheochromocytomas in Asian patients. This prospective study was conducted at Samsung Medical Center between May 2010 and July 2011. The study contained 245 subjects, including 28 patients with histologically-proven pheochromocytoma, 44 with histologically-proven non-pheochromocytoma, 112 controls suspected of having tumors but with negative investigations during two or more years of follow-up, and 45 healthy normotensive volunteers. Plasma-free metanephrines were measured by LC-MS/MS. The cut-off values with optimal sensitivity and specificity for plasma metanephrine and plasma normetanephrine were 0.33 nmol/L and 0.61 nmol/L, respectively. Both the plasma metanephrines measurement and urinary metanephrines measurement had a sensitivity of 96.4% (p = 1.00). However, the urinary metanephrines measurement was significantly more specific than the plasma metanephrines measurement (94.2% vs 75.6%; p < 0.001). When we applied cut-off values based on BMI, specificity improved from 75.6% to 87.2%, with a comparable gain in sensitivity. From a diagnostic perspective, measurement of free metanephrines in plasma drawn in the seated position is highly sensitive but insufficiently specific when compared with measurement of 24-h urinary fractionated metanephrines. The specificity may be improved by applying cut-off values based on BMI. We suggest that free metanephrines in plasma drawn from seated position can also be used as an initial screening test to ensure that pheochromocytomas are not missed in Asian patients.

Key words: Pheochromocytoma, Metanephrine

PHEOCHROMOCYTOMAS are highly heterogeneous chromaffin cell tumors arising in the adrenal glands that should be considered in patients with secondary hypertension or other manifestations of catecholamine excess [1]. Because catecholamine secretion can lead to lethal complications [2, 3], the biochemical tests for pheochromocytomas should have a high diagnostic sensitivity to avoid false negatives. On the other hand, adrenal incidentalomas have become an important clinical entity for which pheochromocytoma must be considered due to the widespread use of imaging studies [4]. Since pheochromocytomas are rare tumors [5-7], the inadequate specificity of biochemical tests is a problem in diagnoses [8].

We previously showed that the cut-off values of 24-h urinary fractionated metanephrines for Koreans were lower than those from European decent [9]. Moreover, different cut-off values of the 24-h urinary fractionated
metanephrines, according to sex, had a higher level of sensitivity and specificity for the diagnosis of pheochromocytoma [9]. However, the measurement of 24-h urinary fractionated metanephrines is inconvenient and cumbersome for patients, and there is a risk of improper collection. Recent studies have reported that the measurement of plasma free metanephrines can detect pheochromocytomas with high sensitivity and specificity, leading to its recommendation as the single biochemical test of choice [10, 11]. However, their conclusions were only valid with cut-off values from blood samples following 20-30 min of supine position period. Whether this requirement is feasible for every patient at every center remains questionable. The clinical utility of plasma free metanephrines as a diagnostic for pheochromocytomas in Asian patients was lately evaluated by Tanaka et al. [12], but the plasma free metanephrines were determined using an enzyme immunoassay. Unfortunately, such procedures can be susceptible to non-specific binding and cross-reactivity leading to lower diagnostic sensitivity and specificity compared to the liquid chromatography-tandem mass spectrometry (LC-MS/MS) which is known a reliable and sensitive test for the biochemical detection of pheochromocytoma [13].

The primary endpoint of this study was to establish cut-off values for plasma free metanephrines in a seated position for detecting pheochromocytoma, and the secondary point was to determine the diagnostic efficacy of plasma free metanephrines in a seated position compared with 24-h urinary metanephrines for detecting pheochromocytomas.

**Patients and Methods**

**Study population**

From May 2010 and through July 2011, we enrolled 245 patients tested for pheochromocytoma at Samsung Medical Center and 45 healthy volunteers. Patients were tested as part of an evaluation of a suspected pheochromocytoma based on episodes (e.g., headache, sweating, palpitation, dyspnea, pallor, or flushing), sustained or paroxysmal hypertension, or adrenal mass on imaging. The Institutional Review Board of Samsung Medical Center approved this study, and all patients provided informed consent.

Patients with pheochromocytoma were confirmed by a pathological examination of the surgically resected or biopsied tumors. Pheochromocytoma was excluded when the pathological examination of a surgically resected adrenal mass determined it was not pheochromocytic, or the lack of pheochromocytoma during the two or more years of follow-up after initial testing. A total of 61 patients who did not meet the criteria for confirmation or exclusion of pheochromocytomas was not included. The patients with confirmed pheochromocytoma (n=28) and those without pheochromocytoma (n=156, control group) were selected for the final analyses. Among the patients with pheochromocytoma, 18 had sporadic and 10 hereditary pheochromocytoma (four with multiple endocrine neoplasia type 2, three with hypersecreting paraganglioma, and three with von Hippel-Lindau syndrome). The patients without pheochromocytoma consisted of 44 histologically-excluded pheochromocytoma patients (32 with adrenal cortical adenoma, four with metastatic renal cell carcinoma, three with adrenal mesothelial cyst, two with endothelial cyst, one with a pseudocyst, one with primitive neuroectodermal tumor, and one with cavernous lymphangioma) and 112 controls with information confirming a lack of pheochromocytoma during at least two years of follow-up (median follow-up, 2.6 years; range, 2.0-3.2 years).

**Biochemical tests**

Patients and healthy volunteers were instructed to fast and abstain from caffeinated beverages and to avoid taking acetaminophen overnight. Blood samples from the patients and healthy volunteers were collected while those were in a seated position without preceding rest on arriving in specimen collection room. Samples of blood were drawn by five experienced medical technologists, and collected, placed on ice and centrifuged within 30 min of collection. Plasma was stored at -70°C and assayed for levels of plasma metanephrines at the end of the study. Twenty-four-hour urinary metanephrines were collected into opaque bottles containing hydrochloric acid to ensure analyte stability. Plasma samples were analyzed by LC-MS/MS. Twenty-four-hour urinary metanephrines were analyzed using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with an ESA Coulochrom III electrochemical detector (ESA, Chelmsford, MA, USA). Different diagnostic cut-off values of 24-h urinary fractionated metanephrines were applied according to sex based on our previous study [9].

**Statistical analysis**

Age, body mass index (BMI), tumor size, and plasma levels of metanephrines expressed as medians (25th,
Plasma metanephrine for pheochromocytoma

75th percentile). Differences in plasma metanephrines levels between groups according to sex or BMI were compared using a Mann-Whitney test. Categorical data were summarized using frequencies, and differences between groups were compared using the χ² test. Receiver-operating-characteristics (ROC) curves were used to assess cut-off values that had the maximal sum of sensitivity and specificity. Because we assumed that cut-off values might differ according to sex or BMI, we determined cut-off values with and without considering sex and BMI. Sensitivity was calculated as the percentage of true-positive over the total of true-positive plus false-negative test results in patients with pheochromocytoma. Specificity was calculated as the percentage of true-negative over the total of true-negative plus false-positive test results in patients without pheochromocytoma. Differences in sensitivities and specificities of plasma and urinary measurements were compared using McNemar’s test. Spearman’s correlation test was used to identify associations between plasma free metanephrines levels and variables including sex, age, hypertension and BMI. Multiple linear regression models were conducted to evaluate the associations between plasma metanephrine and normetanephrine, and variables including sex, age, hypertension, and BMI. All statistical tests were two-sided, and analyses were executed using SAS version 9.3 (SAS Institute, Cary, NC). A p value of less than 0.05 was considered statistically significant.

Results

Baseline clinicopathologic features

Table 1 shows the baseline characteristics of the patients with or without pheochromocytoma and healthy volunteers. Patients with pheochromocytoma were significantly younger (p = 0.002), and presented with larger tumors (p = 0.005), and higher plasma metanephrine (p < 0.001) and normetanephrine (p < 0.001) levels than those without pheochromocytoma. There were no significant differences between patients with or without pheochromocytoma with respect to sex and BMI.

Patients without pheochromocytoma were older than the healthy volunteers (p < 0.001) and presented with higher BMI (p < 0.001). The control group and healthy volunteers were similar with respect to sex and plasma metanephrines.

Reference intervals of plasma metanephrines according to gender and BMI

In Table 2, the medians and 2.5th and 97.5th percentiles for plasma metanephrine and normetanephrine in control group and healthy volunteers are shown. The median plasma concentrations of metanephrine were significantly higher in males (0.22 nmol/L) than in females (0.19 nmol/L) (p = 0.001). However, there was no significant difference in plasma concentrations of normetanephrine between males (0.45 nmol/L) and females (0.39 nmol/L) (p = 0.06).

We further explored the differences in plasma metanephrines according to BMI cut-off points for the overweight in Asian populations (BMI < 23 and BMI ≥ 23) [14]. There was no significant difference in plasma concentrations of normetanephrine between the BMI < 23 group (0.42 nmol/L) and the BMI ≥ 23 group (0.41 nmol/L) (p = 0.46), whereas plasma concentrations of metanephrine were significantly higher in the BMI < 23 group (0.21 nmol/L) than in the BMI ≥ 23 group.

| Table 1 Baseline characteristics of patients and healthy volunteers (N = 229). |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                              | Pheochromocytoma (n=28) | Control (n=156) | Healthy volunteers (n=45) |
| Sex, M/F                                   | 15/13            | 92/64           | 23/22           |
| Age (years)                                 | 44.5 (31.5, 56.3) | 54.5 (47.0, 64.0) | 24.0 (22.0, 25.0) |
| BMI (kg/m²)                                 | 24.8 (21.8, 27.1) | 25.7 (23.0, 27.8) | 20.5 (19.0, 23.6) |
| Tumor size (cm)                             | 3.5 (2.4, 5.1)   | 2.0 (1.5, 3.5)   | NA              |
| Plasma MN (nmol/L)                          | 0.56 (0.22, 2.28) | 0.20 (0.16, 0.25) | 0.21 (0.17, 0.24) |
| Plasma NMN (nmol/L)                         | 3.12 (0.72, 8.09) | 0.42 (0.27, 0.57) | 0.39 (0.32, 0.51) |
| MN, metanephrine; NMN, normetanephrine; NA, not applicable; NS, not significant. |
| a Values are presented as medians (25th, 75th percentile). |
| b The control group included 13 patients with suggestive symptoms or hypertension, but the disease was excluded by negative imaging test and observed at least 2 years of follow up. |
| c A comparison of patients with pheochromocytoma vs. control. |
| d A comparison of control vs. healthy volunteers. |
nephrine sensitivity and specificity were 92.3% and 82.8%, respectively. When considering the combination of different cut-off values of plasma metanephrine and normetanephrine according to sex, the sensitivity (96.4%) was same as upper cut-off values without considering sex differences (96.4%) while the specificity (78.2%) was slightly higher than upper cut-off values without considering sex differences (75.6%).

In addition, we analyzed the diagnostic efficacy of plasma metanephrines according to BMI. The cut-off values for the plasma metanephrine level were 0.43 nmol/L in the BMI <23 group and 0.33 nmol/L in the (0.19 nmol/L) (p = 0.04).

**Diagnostic efficacy of biochemical tests for detection of pheochromocytoma**

The areas under the curves (AUCs) were as follows (Fig. 1): plasma metanephrines, 0.954 [95% confidence interval (95% CI), 0.897-1.000]; 24-h urinary fractionated metanephrines, 0.996 (95% CI, 0.990-1.000); urinary catecholamines, 0.841 (95% CI, 0.724-0.958); urinary dopamine, 0.630 (95% CI, 0.474-0.786); and urine vanillylmandelic acid, 0.819 (95% CI, 0.696-0.942).

The diagnostic efficacy of biochemical tests for detection of pheochromocytoma is shown in Table 3. The established upper cut-offs for both plasma metanephrine (0.50 nmol/L) and normetanephrine (0.90 nmol/L) from the Mayo Clinic [15] yielded a diagnostic sensitivity of 85.7% and a specificity of 94.2% for the detection of pheochromocytoma. A ROC curve indicated that low cut-off points with plasma metanephrine (0.33 nmol/L) and normetanephrine (0.61 nmol/L) in this study provided a higher sensitivity of 96.4% and a lower specificity of 75.6% than applying established upper cut-offs from Mayo Clinic.

We further analyzed the diagnostic efficacy of plasma metanephrines according to sex. The cut-off values for plasma metanephrine level were 0.35 nmol/L in males and 0.33 nmol/L in females, and those for plasma normetanephrine level were 0.64 nmol/L in males and 0.60 nmol/L in females. In male subjects, the plasma metanephrine sensitivity and specificity were 73.3% and 89.1%, respectively; the plasma normetanephrine sensitivity and specificity were 96.7% and 84.8%, respectively. In female subjects, the plasma metanephrine sensitivity and specificity were 61.5% and 90.6%, respectively; the plasma normetanephrine sensitivity and specificity were 92.3% and 82.8%, respectively. When considering the combination of different cut-off values of plasma metanephrine and normetanephrine according to sex, the sensitivity (96.4%) was same as upper cut-off values without considering sex differences (96.4%) while the specificity (78.2%) was slightly higher than upper cut-off values without considering sex differences (75.6%).

In addition, we analyzed the diagnostic efficacy of plasma metanephrines according to BMI. The cut-off values for the plasma metanephrine level were 0.43 nmol/L in the BMI <23 group and 0.33 nmol/L in the
Correlation between sex, age, hypertension, BMI, and plasma metanephrines (Table 4)

We detected a weak positive correlation between age and plasma metanephrine level \((r = 0.132, p < 0.001)\), and between age and plasma normetanephrine level \((r = 0.092, p < 0.001)\). BMI showed a weak negative correlation with the plasma metanephrine level \((r = -0.125, p < 0.001)\) and plasma normetanephrine level \((r = -0.072, p < 0.001)\). In multiple linear regression analysis, sex \((p < 0.001)\), age \((p = 0.010)\), and BMI \((p < 0.001)\) were independently related to the plasma metanephrine level. We also found sex \((p = 0.014)\), hyper-
tension ($p = 0.049$), and BMI ($p = 0.017$) were significantly associated with plasma normetanephrine levels after adjustment.

**Discussion**

In the present study, we confirmed that measurement of plasma free metanephrines offers a high sensitivity for the diagnosis of pheochromocytomas equivalent to that of 24-h urinary fractionated metanephrines. However, plasma free metanephrines taken from a seated position have a low specificity when compared with 24-h urinary fractionated metanephrines.

Previous studies have demonstrated the high diagnostic efficacy of plasma metanephrines, with high sensitivities ranged from 96% to 100% and variable specificities ranged from 85% to 100% [10, 11, 13, 15-19]. The cut-off values for positivity of plasma metanephrines, sampling positions and demographic characteristics of subjects without pheochromocytoma were slightly different among the studies [10, 11, 13, 15-19]. Sawka et al. described cut-off values for positivity were 0.5 nmol/L plasma metanephrine or 0.9 nmol/L plasma normetanephrine based on a reference range established by the Mayo Medical Laboratories [15]. They showed that the plasma metanephrines taken from a seated position was highly sensitive but lacked specificity when compared with 24-h urinary metanephrine and catecholamine levels [15]. On the other hand, Sarathi et al. [18] and Christensen et al. [19] established cut-off values of plasma metanephrines from a seated position according to information from immunoassay kit manufacturers and that showed a high specificity. In studies by Lenders et al. [10], Raber et al. [11] or Vaclavik et al. [16], the criterion for positivity from a supine position was a plasma metanephrine of 0.3 nmol/L and a plasma normetanephrine of 0.6 nmol/L, established by a laboratory reference range [20]. They recommended plasma metanephrines as the best test for excluding or confirming pheochromocytomas [10, 11, 16]. In the current study, the cut-off values of plasma metanephrine and plasma normetanephrine from a seated position with an optimal sensitivity and specificity were 0.33 nmol/L and 0.61 nmol/L, respectively. The cut-off points were similar to previous reports [10, 11, 16], but the cut-points previously used were the upper limit of the normal subjects and patients with hypertension and were derived from blood samples from the supine position. Although the blood samples were drawn from the seated position, the cut-off values of plasma free metanephrines in this study were lower than those of previous studies derived from the seated position [13, 15, 17-19]. Moreover, the cut-off values in the current study provided a higher sensitivity of 96.4% and a lower specificity of 75.6% than applying established upper cut-offs from the Mayo Clinic [15]. Lenders et al. also recommended the use of the upper reference limit determined from blood samples collected in the supine position rather than in the seated position to preserve high diagnostic sensitivity [21]. The present finding of lower cut-off values of plasma free metanephrines providing inadequate specificity may be partly explained by the seated position during blood sample collection and ethnic differences. Because there have been no reports on the differences in plasma metanephrines between seated and supine conditions in non-Western countries, further studies are warranted to confirm this findings.

Pheochromocytomas have been known rare tumors, but can be fatal. In addition, adrenal incidentalomas have reported an increase in incidence due to the widespread use of imaging studies [4] and become an important clinical entity for which pheochromocytoma must be considered. The measurement of 24-h urinary fractionated metanephrines is well-established, widely available, and relatively easy test with accuracy [4]. We previously showed that different cut-off values of 24-h urinary fractionated metanephrines, according to sex, had a sensitivity of 97.1% and a specificity of 91.2% for the diagnosis of pheochromocytoma [9]. However, 24-h urine collections can be inconvenient for patients, which limit its usefulness in outpatient situations. Moreover, there are potential problems with the reliability of incompletely timed urine collections [4]. On the other hand, blood sampling is relatively convenient for patients and reduces the chance of improper collection. Adherence to sampling in the supine position has been emphasized as a condition to minimize false-positive test results [21]. However, whether such condition may not be practical for every patient. Previous study by Lenders et al. [21] carried the financial implications of sampling under both seated and supine positions considering the assumption of a prevalence rate of pheochromocytoma of 0.1%. They suggested plasma free metanephrines in a seated position as an effective test for ruling out pheochromocytoma, and repeat testing with samples in a supine position would offer a cost-effective approach for deal-
Plasma metanephrine for pheochromocytoma

Many cases, further studies are needed to confirm this finding.

Our study has some limitations. First, we cannot be certain that the individuals in the control group did not have a pheochromocytoma without confirming it surgically. Second, we cannot rule out the possibility of false-positive results by some unmeasured factors. Because a seated position during blood sampling was identified as a factor likely to increase the incidence of false-positive results, it is recommended that blood sampling during testing should be carried out in the supine position [4, 24]. However, blood sampling after maintaining the supine position for 20-30 min is not feasible for every patient at every center. For outpatients, blood sampling from a seated position may be more practical. Acetaminophen is known to directly interfere with plasma free metanephrines assays [25]. Dietary influences such as catecholamine-rich products may induce an increase of plasma normetanephrine [24]. Although patients were instructed to fast and abstain from caffeinated beverages and to avoid taking acetaminophen overnight, we lacked information on other exposures. However, we hope that the negative results of 24-h urinary fractionated metanephrines and other biochemical tests sufficiently overcome this limitation. Finally, the reference values used at our institution have not been validated by other institutions.

In summary, our data indicates that plasma metanephrines in the seated position are highly sensitive, but of insufficient specificity, when compared with 24-h urinary fractionated metanephrines. The specificity may be improved if different cut-off values, according to BMI, are applied. Thus, we suggest that plasma metanephrines may be used as an initial screening test to ensure pheochromocytomas are not missed. Future prospective, multicenter trials with a larger sample of patients are required to confirm our findings.

Acknowledgement

This study was supported by the Samsung Medical Center, Clinical Research Development Program grant, #CRS-111-01-1. The authors appreciate the Biostatistics and Clinical Epidemiology Center of Samsung Medical Center for their statistical assistance.

Author Disclosure Statement

The authors have no competing interests to disclose.
References