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Urinary Free Metanephrines for Diagnosis of Pheochromocytoma and Paraganglioma

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Background: Pheochromocytoma and paraganglioma (PPGL) is diagnosed through biochemical confirmation of excessive catecholamines in urine and plasma. Recent technological developments have allowed us to measure urinary free metanephrines; however, the diagnostic accuracy of these new methods and the diagnostic cutoff values have not been evaluated.
Methods: This is a retrospective study of 595 subjects, including 71 PPGL cases and 524 controls. PPGL was based on pathological confirmation. Subjects with no evidence of PPGL over 2 years were included in the control group.
Results: Urinary free metanephrines yielded similar area under the curve (AUC) to urinary fractionated metanephrines and plasma free metanephrines. However, urinary free normetanephrine yielded a better AUC than did urinary fractionated normetanephrine. The optimal cutoff for urinary free metanephrine and normetanephrine corrected for urinary creatinine yielded 97.2% sensitivity and 98.1% specificity.
Conclusion: Urinary free metanephrines are a reliable method for diagnosing PPGL in Asian populations compared with existing biochemical methods.

Keywords: Metanephrine; Normetanephrine; Pheochromocytoma; Paraganglioma

INTRODUCTION

Pheochromocytoma and paraganglioma (PPGL) is a rare tumor that originates in chromaffin cells, which produce catecholamine [1]. PPGL can cause various symptoms, but it is difficult to diagnose because an asymptomatic phenotype also is common [2,3]. Additionally, as the number of imaging tests increases, detection of adrenal incidentaloma increases [4]. Undiagnosed PPGL can be fatal [5], so accurate biochemical testing is needed.

PPGL can be diagnosed by measuring the concentrations of catecholamines and their metabolites of metanephrines and vanillylmandelic acid [2]. Metanephrines in urine are mostly in sulfur-bonded (conjugated) form. However, PPGL has a high proportion of free (unconjugated) forms, especially normetanephrine [6]. Until recently, most centers measured fractionated total (free and sulfur-conjugated form) metanephrines. However, technological developments allow measurement of free

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forms alone [6-8]. A recent study on the efficacy of urinary free metanephrines in a Western population showed high sensitivity [9]. However, the diagnostic accuracy and optimal cutoff value for Asian populations have not been evaluated. In this study, we identified the accuracy and cutoff value of urinary free metanephrines in a Korean sample.

METHODS

We retrospectively reviewed data from patients who were tested for PPGL from August 31, 2013 through March 19, 2019 at Samsung Medical Center (Supplemental Fig. S1). The subjects were those with symptoms of suspected PPGL (e.g., headache, sweating, palpitation, sustained, or paroxysmal hypertension [n=39]) or with an adrenal mass on imaging (n=773). The study protocol was approved by the Institutional Review Board (IRB) of Samsung Medical Center, and the need for informed consent was waived (IRB FILE No. 2020-03-094) because the study was retrospective in design and analyzed de-identified data.

The PPGL group was confirmed by pathological findings from surgically removed tumors. The control group consisted of subjects whose pathologies were confirmed to be non-PPGL (n=71) and who were revealed to have normal adrenal glands upon biochemical and imaging tests over more than 2 years of follow-up (n=453).

Urinary fractionated metanephrines, plasma free and urinary free metanephrines were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). The protocols for measuring metanephrines are the same as previously reported [10,11]. Additionally, when the 24-hour urinary creatinine excretion amount was 1.4 to 2.35 g/day in males or 0.74 to 1.57 g/day in females, the sample was classified as optimally collected according to the reference value of the test reagent.

Receiver operating characteristic (ROC) curves were used to assess cutoff values that yielded the highest sensitivity and specificity. Differences in sensitivities and specificities were compared using McNemar's test. To calculate cutoff values for urinary metanephrines, the combination of urinary metanephrine and normetanephrine that yielded maximal accuracy was estimated using Youden's index method. All statistical tests were two-sided, and analyses were executed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and STATA version 15 (StataCorp., College Station, TX, USA). A P value <0.05 was considered statistically significant.

Table 1. Baseline Characteristics of Patients and Controls

Characteristic	Control (n=524)	PPGL (n=71[a])	P value
Male sex	286 (54.58)	42 (59.15)	0.467
Age, yr	55.00±10.59	53.49±15.13	0.287
BMI, kg/m^2	25.53±3.27	23.49±3.29	<0.001
SBP, mm Hg	129.38±30.35	132.35±18.79	0.422
DBP, mm Hg	80.52±11.74	82.39±15.38	0.227
HTN	237 (45.23)	64 (90.14)	<0.001
DM	144 (27.48)	13 (18.31)	0.100
Serum Cr, mg/dL	0.84±0.20	0.83±0.15	0.728
BUN, mg/dL	14.43±3.91	14.05±4.82	0.455
MDRD eGFR, mL/min/1.73 m^2	93.04±19.70	95.41±20.68	0.345
Urinary free MN, μg/day	16.53±7.91	332.08±801.36	<0.001
Urinary free NMN, μg/day	34.63±143.89	885.48±3,549.64	<0.001
Urinary free MN/Cr ratio, μg/g	14.13±7.87	289.86±738.10	<0.001
Urinary free NMN/Cr ratio, μg/g	29.18±96.41	714.90±2,725.56	<0.001

Values are expressed as number (%) or mean±standard deviation.
PPGL, pheochromocytoma and paraganglioma; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension; DM, diabetes mellitus; Cr, creatinine; BUN, blood urea nitrogen; MDRD, Modification of Diet in Renal Disease; eGFR, estimated glomerular filtration rate; MN, metanephrine; NMN, normetanephrine.
[a]Pheochromocytoma (n=68), paraganglioma (n=3).

RESULTS

After excluding patients who did not meet the study criteria, we included 595 subjects as either confirmed controls or PPGL cases (control n=524, PPGL n=71) (Supplemental Fig. S1). Subject baseline characteristics are shown in Table 1.

When metanephrine and normetanephrine were considered together, plasma and urinary metanephrines all showed high diagnostic performance (Table 2, Supplemental Tables S1, S2). Although urinary free metanephrine showed no significant difference in area under the curve (AUC) compared with other tests for metanephrine, urinary free normetanephrine alone yielded a higher AUC (Table 2). The optimal cutoff for urinary free metanephrine and urinary free normetanephrine had 97.2% sensitivity and 98.1% specificity (Supplemental Table S1).

For cases in which urine was not collected properly, each value was compared with corrected urine creatinine value. Urinary free metanephrine and urinary fractionated metanephrine before or after adjustment for 24-hour urinary creatinine yielded similar AUCs to those in the well-collected 24-hour urine group. However, the AUC of urinary free metanephrine was better after adjustment than before (ROC area 0.728 vs. 0.828, P=0.020) in the poorly collected 24-hour urine group. Urinary fractionated metanephrine did not show any difference in AUC even when calibrated with 24-hour urinary creatinine in the poorly collected group (data not shown).

DISCUSSION

Although analysis of the combined free forms of metanephrine and normetanephrine in urine was not superior to other methods such as those for plasma and urinary fractionated metanephrines, the AUC for urinary free normetanephrine was better than that for urinary fractionated normetanephrine. The lack of statistical difference with combined metanephrine and normetanephrine might be due to the small number of subjects compared with a previous study [9].

Measurement of free metanephrines in urine has advantages

Table 2. AUC Comparison of Each Test Value and Corrected Value with 24-Hour Urinary Cr for Diagnosis of Pheochromocytoma and Paraganglioma

Test	AUC	95% CI	*P* value			
			vs. urinary free MNs[a]	vs. urinary fractionated MNs[a]	vs. plasma free MNs[a]	vs. urinary VMA[a]
Urinary free MN[b]	0.852	0.789–0.915		0.488	0.677	0.006
Urinary fractionated MN[b]	0.863	0.802–0.925	0.488		0.419	0.016
Plasma free MN[c]	0.842	0.779–0.904	0.677	0.419		0.005
Urinary free NMN[b]	0.987	0.978–0.996		0.039	0.231	0.004
Urinary fractionated NMN[b]	0.968	0.945–0.990	0.039		0.903	0.139
Plasma free NMN[c]	0.970	0.939–1.000	0.231	0. 903		0.207
Urinary free MN+NMN	0.978	0.956–1.000		0.202	0.214	0.029
Urinary fractionated MN+NMN	0.989	0.976–1.000	0.202		0.067	<0.001
Plasma free MN+NMN	0.975	0.948–1.000	0.214	0.067		0.017
Urinary free MN/Cr ratio[a]	0.873	0.819–0.926		0.537	0.174	0.142
Urinary fractionated MN/Cr ratio[a]	0.864	0.803–0.925	0.537		0.404	0.112
Urinary free NMN/Cr ratio[a]	0.983	0.972–0.994	-	0.007	0.366	0.000
Urinary fractionated NMN/Cr ratio[a]	0.967	0.948–0.987	0.007		0.887	0.001
Urinary free MN/Cr ratio+NMN/Cr ratio	0.986	0.966–1.000	-	0.570	0.372	0.014
Urinary fractionated MN/Cr ratio+NMN/Cr ratio	0.974	0.966–1.000	0.570	-	0.354	0.008

AUC, area under the curve; Cr, creatinine; CI, confidence interval; MN, metanephrine; VMA, vanillylmandelic acid; NMN, normetanephrine.
[a]MN test results were compared to those of urinary free NM, urinary fractionated MN, or plasma free MN. NMN test results were compared to those of urinary free NNM, urinary fractionated NMN, or plasma free NMN. MN/Cr ratio test results were compared to those of urinary free NM/Cr ratio, urinary fractionated MN/Cr ratio, or plasma free MN. NMN/Cr ratio test results were compared to those of urinary free NNM/Cr ratio, urinary fractionated NMN/Cr ratio, or plasma free NMN; [b]μg/day; [c]nmol/L.

over that of fractionated metanephrines based on their different structures and metabolic processes. The free form of metanephrine is transformed into sulfate-conjugated metanephrine by the action of a specific sulfotransferase isoenzyme, monoamine-preferring sulfotransferase (SULT1A3) [6]. Because SULT1A3 is found in high concentration in the gastrointestinal tract, the level of conjugated metanephrines is affected by food in the gastrointestinal tract [6]. Therefore, urinary free metanephrines can be more useful than urinary conjugated metanephrines in conditions without restriction of diet [12,13]. In addition, while conjugated metanephrines in urine require an acid hydrolysis step [6,14], the free form of metanephrines in urine does not undergo this process, reducing analytical interference and showing higher specificity [10,14].

In this study, urinary fractionated metanephrines had high sensitivity and specificity, as previously reported [13,15,16], as did urinary free metanephrines. In addition, as with urinary fractionated metanephrines [17,18], urinary free metanephrines show higher diagnostic accuracy due to adjustment for urinary creatinine when urine collection is poor. In this study, when urinary free metanephrine was poorly collected, the AUC increased after correcting the measurement for urinary creatinine.

Measurement for urinary free metanephrines has several strengths compared to that for plasma free metanephrines. The sensitivity and specificity of plasma free metanephrines were high, ranging from 96%–100% and 85%–100%, respectively [3,10,19]. However, when measured in a sitting position, the specificity of plasma free metanephrines decreases [11,20]. Considering that most adrenal incidentalomas are examined in outpatient clinics, measurement for urinary free metanephrine that is not affected by posture would be more useful.

It also has been reported that plasma free metanephrines are affected by other diseases [7,15]. Plasma free metanephrines have been found to be elevated in hypothyroidism due to increased norepinephrine secretion, while urinary free metanephrines were not affected [7,15]. As plasma free metanephrines can be extracted by extraneuronal uptake in tissues and metabolized by sulfuric conjugation before being excreted into urine, free metanephrines are less affected in urine than in blood [16].

This study has some limitations. First, it did not include healthy volunteers. Second, the control group included patients who were less likely to develop PPGL after more than 2 years of follow-up. Therefore, people who had not undergone surgery could not be definitively ruled out for PPGL. Third, our study enrolled a small number of subjects who were all analyzed at only one center. To confirm the clinical superiority of urinary free metanephrines measurement, further studies with a larger number of subjects are needed. Nevertheless, it is significant that this study was the first to validate a urinary free metanephrine test and estimate a diagnostic cutoff value for an Asian population.

In summary, urinary free normetanephrine had higher diagnostic efficacy than urinary fractionated normetanephrine, although combined urinary free metanephrine and normetanephrine did not show higher diagnostic accuracy compared to plasma free or urinary fractionated metanephrines.

CONFLICTS OF INTEREST

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

AUTHOR CONTRIBUTIONS

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

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