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Original Article

Plasma metanephrine and normetanephrine measured by LC-MS/MS in the diagnosis of pheochromocytoma and paraganglioma

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Abstract: Purpose: The aim of this study was to evaluate the diagnostic value of plasma metanephrine (MN) and normetanephrine (NMN) in pheochromocytoma and paraganglioma (PPGL) by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Methods: We selected patients with indications of PPGL screening in Zhongshan Hospital affiliated to Fudan University from September 2013 to March 2015. The plasma NMN and MN levels were measured by LC-MS/MS, and were compared between patients with PPGL and those without. Receiver operating characteristic (ROC) curves were utilized to evaluate the sensitivity and specificity of plasma MN and NMN in the diagnosis of PPGL. Results: This study consisted of 906 patients without PPGL and 58 patients with PPGL, including 47 patients with pheochromocytoma and 11 patients with paraganglioma. The area under the ROC curve was 0.789±0.039 for MN and 0.975±0.015 for NMN. When the cut-off value of plasma MN was 80.7 pg/ml, the sensitivity and specificity were 56.9% and 94.7%, respectively. Additionally, the sensitivity and specificity were 91.4% and 99%, respectively, for a plasma NMN cut-off value of 173.9 pg/ml. For a combination of plasma MN and NMN, the sensitivity and specificity were 96.6% and 93.8%, respectively. Conclusions: Plasma MN and NMN play an important role in the diagnosis of pheochromocytoma and paraganglioma by LC-MS/MS.

Keywords: Metanephrine, normetanephrine, pheochromocytoma, paraganglioma

Introduction

A pheochromocytoma is a tumor originating from adrenal medullary chromaffin tissue, which produces and secretes one or more catecholamines, such as epinephrine, norepinephrine and dopamine. A paraganglioma is a tumor arising from extra-adrenal chromaffin cells or tissues located at the paravertebral sympathetic ganglion of thorax, abdomen and pelvis [1]. However, it commonly produces a type of catecholamines, although some may not.

Approximately 0.2% to 0.6% of patients with hypertension have been found to have a pheochromocytoma or paraganglioma [2, 3]. The prevalence of pheochromocytoma in patients with adrenal incidentaloma is approximately 5% [4]. Nearly 85% of chromaffin-cell tumors are pheochromocytomas, whereas 15% are paragangliomas [5]. At least one-third of patients with PPGLs carry germline mutations that present with multifocal disease and at a younger age compared to those with sporadic cases [6].

Because of the paroxysmal or persistent secretion of catecholamines, the clinical manifestations of patients with PPGLs are headache, palpitation, sweatiness, and paroxysmal or sustained hypertension, which can cause serious or even life-threatening heart, brain and kidney complications [7]. The tumor size increases with time, leading to tumor invasion or metastasis to other tissues or organs. Additionally, because pheochromocytoma and paraganglioma belong to a class of hereditary disease, the discovery of a proband requires the diagnosis and treatment of other family members as soon as possible.

The Endocrine Society clinical practice guidelines of pheochromocytoma and paraganglioma recommend that initial biochemical testing

for PPGL should include measurements of plasma free metanephrines or urinary fractionated metanephrines [1]. Metanephrines (MNs) consist of metanephrine (MN) and normetanephrine (NMN), which are 3-O-methylated metabolites of epinephrine and norepinephrine, respectively. In this study, we measured the plasma MN and NMN levels by liquid chromatography-tandem mass spectrometry. Therefore, the aim of this study was to evaluate the diagnostic value of the plasma MN and NMN concentrations in patients with PPGLs by using LC-MS/MS.

Materials and methods

Study population

Patients with indications of PPGL after screening were selected in Zhongshan Hospital affiliated to Fudan University from September 2013 to March 2015. The clinical settings for PPGL testing included the signs and symptoms of PPGL (hypertension, headache, palpitation, sweatiness or hypermetabolic state), particularly if paroxysmal; PPGL symptoms provoked by use of posture change or medications associated with adverse effects; adrenal incidentaloma and retroperitoneal tumor, with or without hypertension; and a previous history of PPGL. However, the patients with head and neck PGLs, PPGLs without surgery or metastatic PPGLs were excluded. Written consent was obtained from all patients.

The diagnosis of a pheochromocytoma or paraganglioma was histologically confirmed in all patients. The control groups were primary hypertension cases as assessed by clinical diagnosis and secondary hypertension patients caused by other factors, including primary aldosteronism and Cushing's syndrome. Patients with non-functioning adrenal tumors and no surgical indications were followed up for at least six months to exclude PPGL by examining the plasma MNs and in imaging tests.

Sample preparation

To draw blood in the supine position, patients were fully recumbent for at least 15 minutes before sampling. Venous blood was collected into EDTA anticoagulant tubes. Samples were centrifuged for ten minutes under the speed of 3000 r/min, and the plasma was then transferred to opaque tubes and frozen immediately. Subjects were informed to avoid variable interference with analytical methods or pharmacological effects on the disposition of catecholamines, such as tricyclic antidepressants, phenoxybenzamine, paracetamol, levodopa, sympathomimetics and coffee. Pharmacological interference was avoided by the withdrawal or substitution of drugs causing false-positive results for at least a week. Additionally, blood was collected after overnight fasting to prevent diets from affecting the plasma measurements [8].

Analytical methods

Sample analysis was performed on a Waters® Xevo™ TQ MS ACQUITY UPLC® System, and plasma NMN and MN levels were measured by liquid chromatography-tandem mass spectrometry. These methods were set up by the department of clinical laboratory medicine in Zhongshan Hospital affiliated with Fudan University [9].

The intra-assay coefficients of variation (CVs) for the different plasma concentrations ranged from 4.7% to 5.3% for MN and 4.9% to 6.2% for NMN. The inter-assay coefficients of variation ranged from 5.2% to 7.1% for MN and 5.1% to 6.7% for NMN. The lower detection limit of the method was 20 pg/ml.

A true-positive test was an elevation equal to or higher than the upper reference limit (URL) for either or both measurements in patients with histologically confirmed PPGLs. A true-negative result was defined as values lower than the URL for both metabolites in patients without PPGLs. The sensitivity of each biochemical measurement was estimated from the percentage of true-positive results among the total of the true-positive and false-negative results for patients with PPGLs. The specificity of each biochemical measurement was estimated from the percentage of true negative results among all of the true-negative and false-positive results for patients without PPGLs [10]. ROC curves showed the relative changes in sensitivity (Y axis) and 1-specificity (X axis) for the diagnosis of PPGL. Cut-off points were determined according to the maximum sum of sensitivity and specificity.

Statistical analysis

Statistical analyses were carried out using SPSS software package version 18.0. Mea-

Table 1. Patient characteristics and plasma concentrations of metanephrines

	Confirmed PPGL	PPGL excluded	*p* value
N	58	906	
Age	47.72±13.43	50.96±14.95	NS
Sex (F/M)	33/25	434/472	NS
Metanephrine (pg/ml)	100.4 (35.2-383.3)	34.0 (22.2-49.2)	<0.001
Normetanephrine (pg/ml)	1472.2 (745.7-2901.5)	76.4 (55.0-102.8)	<0.001

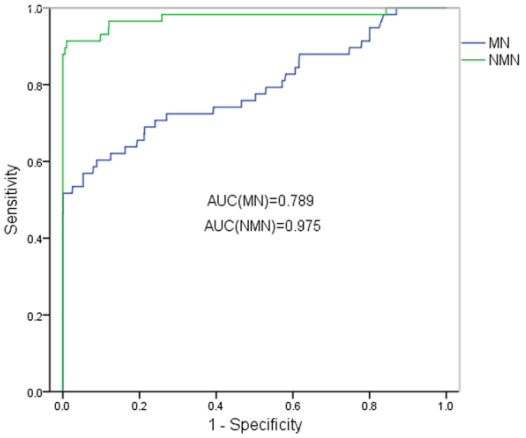

Figure 1. ROC curve analysis for plasma MN and NMN by LC-MS/MS. ROC curves for comparing the test performance of plasma MNs measured by LC-MS/MS. The blue line gives the of MN characteristics in the diagnostic performance, and the green line shows the plasma NMN AUC.

surement data were presented as the mean ± SD and compared using the independent sample *t*-test. If the data were not normally distributed, they were displayed as medians with interquartiles, and the Mann-Whitney *U* test was employed. The Wilcoxon test was performed to compare the plasma MNs before and after the operation. ROC curves were utilized to evaluate the sensitivity and specificity of plasma MN and NMN in the diagnosis of PPGL. Pearson's correlation analysis was used to examine the relationships between the tumor diameters and the plasma concentrations of metanephrines. Statistical significance was set at P<0.05.

Results

Patient characteristics

In this study, 964 patients were selected from September 2013 to March 2015. Of these, 58 patients were histologically confirmed to have PPGL, including 47 patients (81%) with pheochromocytoma and 11 patients (19%) with paraganglioma. The other 906 patients contained primary hypertension cases, secondary hypertension patients caused by other factors and non-functioning adrenal tumors that were assessed by clinical or pathological diagnosis to exclude pheochromocytoma. **Table 1** shows the characteristics of all patients included in the study. There were no significant differences in age or sex at baseline.

Diagnostic test performance of plasma MNs

The plasma concentrations of MN were 100.4 (35.2-383.3) pg/ml and 34.0 (22.2-49.2) pg/ml in patients with and without PPGLs, respectively. The plasma concentrations of NMN, respectively were 1472.2 (745.7-2901.5) pg/ml and 76.4 (55.0-102.8) pg/ml in patients with and without PPGLs. The mean plasma MN and NMN concentrations in patients with PPGLs were significantly higher than in those without (both P<0.05), as shown in **Table 1**. The area under the ROC curve was 0.789±0.039 for MN and 0.975±0.015 for NMN (**Figure 1**). When the plasma MN cut-off value was 80.7 pg/ml, the sensitivity and specificity were 56.9% and 94.7%, respectively. Additionally, the sensitivity and specificity were 91.4% and 99% for the plasma NMN of 173.9 pg/ml, respectively. For the combination of MN and NMN, the sensitivity and specificity were 96.6% and 93.8%, respectively (**Table 2**).

Preoperative and postoperative plasma MN concentrations in patients with PPGLs

In the 26 patients with PPGLs, the MN plasma concentrations were 92.1 (30.2-361.1) pg/ml before surgery and 24.5 (20.7-45.3) pg/ml after surgery. The NMN plasma concentrations were 1167.4 (462.3-2572.0) pg/ml and 78.0 (52.7-104.3) pg/ml in patients with PPGLs before and after surgery, respectively. The mean plasma MN and NMN concentrations in the 26 patients with PPGLs after operation were significantly lower than those before operation (both P<0.05).

Table 2. Test characteristics of plasma NMN, MN and a combination of both in the diagnosis of PPGL

	Upper reference limit	Sensitivity % (n/N)	Specificity % (n/N)
Normetanephrine (NMN)	173.9 pg/ml	91.4% (53/58)	99% (897/906)
Metanephrine (MN)	80.7 pg/ml	56.9% (33/58)	94.7% (858/906)
Combined metanephrines	173.9 pg/ml and/or 80.7 pg/ml	96.6% (56/58)	93.8% (850/906)

Combined metanephrines were defined as positive if the value of either NMN or MN was above the upper reference limit.

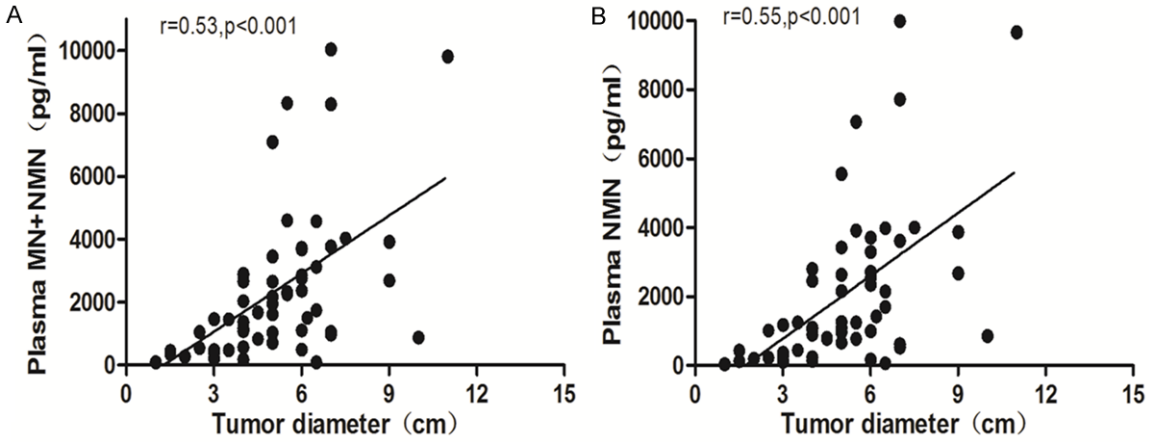

Figure 2. Relationships between tumor diameters and plasma concentrations of MNs (A) or NMN (B). Pearson's correlation analysis indicated that the tumor diameters showed positive relationships with total plasma MNs (A) and NMN (B).

The relationship between plasma MNs and tumor size

The mean tumor diameter of the 58 patients with PPGLs was 5.1±2.0 cm. There was a positive correlation between the tumor diameters and the total plasma MN and NMN concentrations (r=0.53, P<0.001) (**Figure 2**). Tumor sizes also showed positive relationships with plasma NMN (r=0.55, P<0.001), but no differences with plasma MN were observed.

Discussion

Initial biochemical tests for PPGL contained measurements of plasma catecholamines, urinary catecholamines, urinary vanillylmandelic acid, plasma metanephrines or urinary fractionated metanephrines. As demonstrated in a previous report [10], the sensitivities of plasma metanephrines (99%) and urinary fractionated metanephrines (97%) were higher than those of plasma catecholamines (84%), urinary catecholamines (86%), and urinary vanillylmandelic acid (64%). Specificity was highest for urinary vanillylmandelic acid (95%); intermediate for plasma metanephrines (89%), urinary catecholamines (88%), and plasma catecholamines (81%); and lowest for urinary fractionated metanephrines (69%). Although the sensitivity of urinary fractionated MNs was similar to that of plasma MNs, their specificity was much lower than other methods [11]. In our study, we evaluated the diagnostic test performance of plasma metanephrines in PPGL and found high sensitivity (96.6%) and specificity (93.8%) values for plasma MNs, which were consistent with previous literature [12]. Therefore, it is recommended that plasma MNs be measured in patients with suspected PPGLs.

As a way to uncovering PPGL, plasma MNs have the following advantages. First, metanephrines are produced within adrenal chromaffin cells by catecholamine O-methyltransferase. Thus, the secretion of these metabolites is independent of exocytotic catecholamine release, which, for some tumors, occurs at low rates or is sporadic in nature [13]. Second, plasma MNs are produced continuously within tumors and have a longer half-life and higher stability than catecholamines do [1]. Third, plasma MN measurements are relatively slightly affected by posture changes or diets, which are only relevant when

these measurements include the dopamine metabolite 3-methoxytyramine [14].

Plasma metanephrines may be measured using enzyme immunoassay (EIA), liquid chromatography with electrochemical detection (LC-ECD), or liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods. Previous evidence has indicated that LC-MS/MS offered not only superior precision compared to immunoassays but also greater accuracy in terms of underestimating the plasma concentrations of MNs [15]. Accumulating evidence has shown that LC-MS/MS has several advantages over LC-ECD. First, the former requires smaller plasma samples, simpler sample preparation and relative freedom from analytical interference. Second, it may reduce the time for sample detection and instrument and reagent costs [16, 17]. This study for the first time used LC-MS/MS in China to measure plasma MNs and to evaluate their diagnostic test performance. We found that this method may improve the accuracy, shorten the testing time and save costs.

The plasma MN and NMN in 26 patients with PPGLs after surgery were significantly lower than those before surgery, and the patients' clinical symptoms mostly disappeared. Our results suggested that the plasma MNs in patients with benign PPGLs could drop to the normal ranges after the surgical removal of the tumor. In the report by Osinga et al. [18, 19], 6%-16% of the observed tumors recurred within 10 years postoperatively during long-term follow-up. Therefore, the close monitoring of plasma MNs in these patients may contribute to the earlier detection of tumor recurrence, whereas patients with MN concentrations within the reference intervals could be spared additional imaging [20].

Previous research has found that the relationships between tumor diameter and plasma or urinary deconjugated metanephrines were stronger than those between tumor diameter and plasma or urinary catecholamines [21]. In our study, the tumor diameters showed positive relationships with total plasma MNs. Furthermore, there was a positive correlation between tumor diameters and plasma NMN. Therefore, the detection of plasma MN and NMN may provide information about tumor size.

Our study has some limitations. Because of the retrospective nature of this study, not all of the data are available. A few patients with nonfunctioning adrenal tumors and no surgical indications had diagnoses of pheochromocytoma were excluded based on clinical diagnosis, but tumors could not always be completely excluded in the absence of pathological diagnosis. Furthermore, the duration of the follow-ups was short.

In conclusion, plasma MN and NMN provide a good test for the exclusion or confirmation of PPGL and should be the first choice of test for tumor diagnosis. Plasma metanephrines are measured more quickly, simply and accurately when using LC-MS/MS, thus contributing to early diagnosis, curative effect evaluation and postoperative follow-up in patients with pheochromocytoma and paraganglioma.

Disclosure of conflict of interest

None.

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