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Indexing of Urinary Catecholamines and Metanephrines by Urinary Creatinine Levels in the Diagnosis of Phaeochromocytoma

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Abstract: The study involved the investigation of the diagnostic utility of 24 h urinary excretion of catecholamines (epinephrine, norepinephrine and dopamine) and metanephrines (normetanephrine and metanephrine) and to compare with catecholamine-to-creatinine and metanephrine-to-creatinine ratio in the diagnosis of phaeochromocytoma. Retrospective study of 116 patients of which 4 had histologically confirmed phaeochromocytoma and 112 patients with essential hypertension. All measurements were performed using High Pressure Liquid Chromatography (HPLC) with Electrochemical Detection (ED) and the urine creatinine concentrations were measured by the Jaffe reaction. The four patients with phaeochromocytoma had mean 24 h urinary excretion rates of 1682.75±175.37 nmol/24 h for epinephrine (vs 54.38±4.61 nmol/24 h in hypertensive group), 16389.25±1295.22 nmol/24 h for fractionated normetanephrine (vs 2050.85±141.57 nmol/24 h in hypertensive group) and 21484.25±3882.60 nmol/24 h for fractionated metanephrine (vs 660.71±50.74 nmol/24 h in hypertensive group). There was excellent sensitivity and specificity for 24 h urinary excretion of fractionated metanephrine (100 and 92.9%) and normetanephrine (100 and 98.2%) for the diagnosis of phaeochromocytoma. The specificity of the metanephrine-to-creatinine ratio was 99.1% compared with 92.9% for fractionated metanephrine. Urinary excretion rate of normetanephrine and metanephrine-to-creatinine ratio are good discriminators of patients with essential hypertension and phaeochromocytoma with the latter test showing better specificity and positive predictive value compared with urinary excretion rate of fractionated metanephrine concentration.

Key words: Metanephrine, normetanephrine, phaeochromacytoma, creatinine, epinephrine

Introduction

Phaeochromocytoma is a catecholamine secreting tumour that arises from the chromaffin cells of the sympathoadrenal system and is a rare cause of secondary hypertension. Its diagnosis is important because the hypertension is usually curable by resection of the tumour, whereas the condition is lethal if untreated (Bravo and Gifford, 1984). The most common associated clinical findings of phaeochromocytoma are sustained or paroxysmal hypertension, headache, palpitations and diaphoresis (Pacak *et al*., 2001) but many, especially those found during screening of patients with multiple endocrine neoplasia type 2 (MEN 2) and von Hippel-Lindau syndrome (VHL) are clinically silent (Eisenhofer, 2001).

The biochemical tests for phaeochromocytoma are based on detecting abnormally increased levels of several different free catecholamines (epinephrine, norepinephrine and dopamine) or their

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metabolites in urine or blood (Bravo, 1994) and are routinely used in the evaluation of hypertensive patients. Measurement of urinary excretion of catecholamines or their metabolites have long represented the gold standards for biochemical detection of phaeochromocytoma (Rosano *et al*., 1991). Urinary free epinephrine, free norepinephrine, total (i.e., free plus conjugated) metanephrine and total normetanephrine when used in combination are considered the most sensitive and specific biochemical tests for the detection of the tumour (Peaston *et al*., 1996). A search of the literature revealed that most published laboratory methods for metanephrine and normetanephrine analysis in urine use high pressure liquid chromatography (HPLC) (Gupta, 1990). HPLC-based techniques for the measurement of catecholamines and metanephrines (normetanephrine and metanephrine) offer a more practical approach than Gas Chromatography (GC) or fluorimetric procedures as uniform HPLC conditions can be utilized for both urinary catecholamines and metanephrines (Parker *et al*., 1986).

There have been studies that have demonstrated that measurement of urinary metanephrine concentrations is more sensitive than measurement of urinary vanillymandelic acid (VMA) or plasma catecholamine concentrations (Gitlow *et al*., 1970; Manu and Runge, 1984). A common problem associated with the measurements of catecholamine or metanephrines is the incompleteness and inconvenience associated with 24 h urine collections. The urinary excretion of metanephrine is either under- or overestimated when the period of urine collection is shorter or longer than twenty four hours. The resulting under estimation leads to false-negative results and the overestimation leads to false-positive results. The diagnosis of phaeochromocytoma using urinary epinephrine, norepinephrine, fractionated metanephrine or fractionated normetanephrine could be improved by correcting with urinary creatinine concentrations. The objective of the study is to investigate whether the 24 h urinary excretion rate of the catecholamines and metanephrines can be improved the diagnosis of phaeochromocytoma compared with the 24 h urinary catecholamine-to-creatinine and metanephrine-to-creatinine ratio.

Materials and Methods

Four patients, 2 women and two men (32-59 years old), with histologically proven phaeochromocytoma were retrospectively studied. All four patients had solely adrenal tumours. One hundred and twelve patients with essential hypertension served as a reference group. All patients were evaluated by clinical history assessment and 24 h urinary collections for measurement of free catecholamines and their metabolites. Positive results of biochemical analysis prompted further study by imaging techniques. The diagnosis of a phaeochromocytoma was made only when confirmed by pathologic analysis of a resected specimen. In patients with slightly increased catecholamine and metanephrine excretion, the absence of phaeochromacytoma was documented by negative results of retesting or negative findings on one or more of the following imaging procedures: adrenal ultrasonography, computed tomography of the abdomen and pelvis, digitalized angiography and whole-body metaido-benzyl guanidine (MIBG) scintigraphy. All studies were done according to the ethical guidelines of the Royal Brompton and Harefield NHS Trust ethics committee and written consent was obtained from each patient.

Urine samples (24 h) were collected in polyethylene containers with 20 mL of 6 mol L^{-1} HCl used as a preservative and stored at 4°C and assays were done within 2 weeks. From 5 days before sampling, patients had dietary restrictions of chocolate, coffee, bananas, vanilla-containing foods, citrus fruits and drugs such as aspirins, α-methyldopa and propranolol.

Urinary catecholamines and metanephrines were measured in all the samples by reverse phase, ion pair HPLC with electrochemical detection (Peaston, 1988). Urine samples for fractionated normetanephrine and metanephrine measurements were acid-hydrolyzed before isolation by a two-step ion exchange purification scheme involving cation and anion exchange resins (Bio-Rad laboratories, Watford, UK). Isocratic HPLC conditions (Peaston, 1988) with electrochemical detection was used

for fractionated metanephrine and normetanephrine analyses. The interassay coefficient of variance for all analyses was less than 8%. Urine creatinine concentrations were measured by the Jaffe reaction (Taussky, 1956) in an acidified 24 h urine sample.

The data was entered into the computer and managed by using SPSS for windows (11.5; SPSS Inc, Chicago). Results were expressed as Mean±SD. The Student's t-test and/or the F-test (analysis of variance) were used to evaluate the probability of any significance between the different biochemical parameters between groups. Diagnostic specificity and sensitivity were calculated using standard formulae (Gallen and Peters, 1986). p-values <0.05 were considered to be statistically significant.

Results

Biochemical tests with measurement exceeding the upper limit of the normal range were regarded as being positive. The upper reference limit was defined as 97.5th percentile calculated from logarithmically transform individual values data from normotensive subjects as well as patients with essential hypertension (non-phaeochromocytoma). The 97.5th percentile was calculated from the antilogarithm of the mean±2 SD of the transformed data (Table 1).

The study group consisted of 116 patients of which 4 had histologically confirmed phaeochromocytoma. These four patients had mean 24 h urinary excretion rates of 1682.75±175.37 nmol/24 h for epinephrine, 1668.00±200.79 nmol/24 h for norepinephrine, 2275.50±60.49 nmol/24 h for dopamine, 16389.25±1295.22 for fractionated normetanephrine and 21484.25±3882.60 nmol/24 h for fractionated metanephrine (Table 1). All the urinary excretion rate of catecholamine except dopamine were significantly elevated above the diagnostic cut-off value with a 2.7 fold increase for norepinephrine and 9.8 fold increase for epinephrine. Likewise the 24 h urinary excretion of fractionated metanephrine and normetanephrine concentrations were significantly elevated above the cut-off value with fold increase ranging from 3.2 for normetanephrine to 12.3 for metanephrine ($p<0.05$; Table 1). The analyte-to-creatinine ratio for the catecholamines and metanephrines are given in Table 2. The epinephrine-to-creatinine and metanephrine-to-creatinine ratios were elevated by 7.9 and 5.5 fold respectively above the diagnostic cut-off values. The individual values of the catecholamines and fractionated metanephrines for the four patients with histologically confirmed phaeochromocytoma are given in Table 3.

Table 1: Mean 24 h excretion of catecholamines and fractionated metanephrines in the urine of essential hypertensive patients and those with histologically confirmed phaeochromocytoma

24 h urinary excretion of analyte (nmol/ 24 h)	Essential hypertensive patients	Patients with histologically confirmed phaeochromocytoma	p-value
Epinephrine	54.38±4.61	1682.75±175.37	0.010
Norepinephrine	310.12±14.90	1668.00±200.79	0.003
Dopamine	1366.98±60.98	2275.50±60.49	0.367
Normetanephrine	2050.85±141.57	16389.25±1295.22	0.015
Metanephrine	660.71±50.74	21484.25±3882.60	0.010

Table 2: Mean analyte-to-creatinine ratio in the urine of essential hypertensive patients and those with histologically confirmed phaeochromocytoma

Analyte-to-creatinine ratio	Essential hypertensive patients	Patients with histologically confirmed phaeochromo-cytoma	p- value
Epinephrine	0.006±0.008	0.165±0.128	0.006
Norepinephrine	0.033±0.034	0.154±0.189	0.001
Dopamine	0.147±0.165	0.217±0.185	0.050
Normetanephrine	0.260±0.455	1.565±0.312	0.015
Metanephrine	0.066±0.121	1.680±0.520	<0.0001

Table 3: The 24 h urinary excretion of catecholamines and metabolites and analyte-to-creatinine ratio in patients with histologically confirmed phaeochromocytoma

	24 h urinary excretion (nmol/24 h)					Analyte-to-creatinine ratio ($\times 10^{-3}$)				
Patient No.	E	NE	D	ME	NMT	E	NE	D	ME	NMT
1	1180	1138	2178	13449	13417	165	159	304	1875	1871
2	1721	1578	2272	27686	17199	151	138	199	1507	1507
3	1856	1942	2447	28573	28573	169	176	222	2590	1399
4	1974	2014	2205	16229	16229	240	240	268	1974	2373

E-Epinephrine; NE-Norepinephrine; D-Dopamine; ME-Metanephrine, NMT-Normetanephrine

Table 4: Sensitivity and specificity of 24 h urinary excretion of catecholamines and metanephrines and analyte-to-creatinine ratio for catecholamines and metabolites in patients with and without phaeochromocytoma

Criterion	Patients with true positive results/patients with pheochromocytoma	Sensitivity (%)	Patients with true negative results/patients without pheochromocytoma	Specificity (%)
Epinephrine ≥149 nmol/24 h	4/4	100	110/112	98.2
Norepinephrine ≥626 nmol/24 h	4/4	100	108/112	96.4
Dopamine ≥2658 nmol/24 h	0/4	0	108/112	96.4
Metanephrine ≥5047 nmol/24 h	4/4	100	104/112	92.9
Normetanephrine ≥1735 nmol/24 h	4/4	100	110/112	98.2
Epinephrine-to creatinine-ratio ≥0.022	4/4	100	108/112	96.4
Norepinephrine-to creatinine-ratio ≥0.101	4/4	100	107/112	95.5
Dopamine-to creatinine-ratio ≥0.477	0/0	0	105/112	93.8
Metanephrine-to creatinine-ratio ≥0.308	4/4	100	111/112	99.1
Normetanephrine-to creatinine-ratio ≥1.169	4/4	100	108/112	96.4

The operating characteristics of the measurements of urinary catecholamines and their electrolytes are shown in Table 4. Based on the cut-off limits for essential hypertensive patients, the sensitivity of 24 h urinary excretion and analyte-to-creatinine ratio of all the catecholamines and fractionated metanephrine and normetanephrine except dopamine was 100%. The specificity of the 24 h urinary excretion of catecholamines measurements for the detection of phaeochromocytoma in all four patients with histologically confirmed phaeochromocytoma ranged from a low of 96.4% for norepinephrine to a high of 98.2% for the use of epinephrine measurements. Likewise, the 24 h urinary excretion rate for metanephrines ranged from a low of 92.9% for metanephrine to a high of 98.2% for the use of normetanephrine measurements (Table 4).

Specificities of the analyte-to-creatinine ratio of the catecholamine metabolites for the detection of histologically confirmed phaeochromocytoma in all four patients were highest for fractionated normetanephrine at 96.4 and 99.1% for fractionated metanephrine, respectively (Table 4). Of the three catecholamine measurements used in the diagnosis of phaeochromocytoma, epinephrine has the highest positive and negative predictive values of 66.7 and 100%, respectively. The corresponding positive and negative predictive values for fractionated metanephrine were 66.7 and 100%, respectively.

The use of the analyte-to-creatinine ratio did not improve the diagnostic sensitivity compared with the use of the 24 h urinary excretion rate for epinephrine, norepinephrine and fractionated normetanephrine. There were decreases in specificity for norepinephrine from 96.4 to 95.5% and 98.2 to 96.4% for epinephrine and fractionated normetanephrine. However, there was an increase in the diagnostic specificity for fractionated metanephrine from 92.9 to 99.1%. Fractionated normetanephrine measurement was determined to be the best discriminator of 24 h the urinary excretion rates in distinguishing essential hypertension and phaeochromocytoma with sensitivity of 100%, specificity of 98.2% and best Negative Predictive Value (NPV) of 100% and Positive Predictive Value (PPV) of 66.7% (Table 5). The metanephrine-to-creatinine ratio was the best discriminator of the analyte-to-creatinine ratio with sensitivity of 100%, specificity of 98.2% and best NPV (100%) and PPV (80%).

Table 5: Negative and positive predictive values of 24 h urinary excretion of catecholamines and metanephrines and analyte-to-creatinine ratio for catecholamines and metabolites in patients with and without phaeochromocytoma

Criterion	Patients with true-positive results/patients with any positive results	Positive predictive value (%)	Patients with true-negative results/patients with any negative results	Negative predictive value (%)
Epinephrine ≥149 nmol/24 h	4/6	66.7	110/110	100.0
Norepinephrine ≥626 nmol/24 h	4/8	50.0	108/108	100.0
Dopamine ≥2658 nmol/24 h	0/4	0.0	108/112	96.4
Metanephrine ≥5047 nmol/24 h	4/12	33.3	104/104	100.0
Normetanephrine ≥1735 nmol/24 h	4/6	66.7	110/110	100.0
Epinephrine-to creatinine-ratio ≥0.022	4/8	50.0	108/108	100.0
Norepinephrine-to creatinine-ratio ≥0.101	4/9	44.4	107/107	100.0
Dopamine-to creatinine-ratio ≥0.477	0/7	0.0	105/105	100.0
Metanephrine-to creatinine-ratio ≥0.308	4/5	80.0	111/111	100.0
Normetanephrine-to creatinine-ratio ≥1.169	4/8	50.0	108/108	100.0

Discussion

In this study we demonstrated excellent sensitivity and specificity for 24 h urinary excretion of fractionated metanephrine (100 and 92.9%) and normetanephrine (100 and 98.2%) for the diagnosis of phaeochromocytoma. The specificity and sensitivity for 24 h urinary excretion of catecholamines such as epinephrine and norepinephrine were also excellent with values of 100 and 98.2% for the former and 100 and 96.4% for the latter. The 24 h urinary excretion of the catecholamines and their metabolites were normalized by using the 24 h creatinine concentration. The study showed that only the specificity of the urinary metanephrine-to-creatinine ratio improved, from 92.9 to 99.1% compared to slight decreases in specificity for the normetanephrine-to-creatinine and epinephrine-to-creatinine ratios (98.2 to 96.4%) and norepinephrine-to-creatinine ratio (96.4 to 95.5%). The urinary normetanephrine excretion was found to be the best discriminator between essential hypertension and phaechromocytoma with a positive predictive value of 66.7% and a negative predictive value of 100%. Urinary metanephrine-to-creatinine ratio had the best positive and negative values of 80 and 100% respectively. Thus the 24 h urinary excretion of normetanephrine and the metanephrine-to-creatinine ratio were the most reliable for diagnosing and excluding phaeochromocytoma.

Several studies have reported that the biochemical demonstration of excessive catecholamine production and its metabolites are essential in the initial diagnosis of phaeochromocytoma and the biochemical tests are based on their measurements in plasma or urine (Peaston *et al*., 1996; Sawka *et al*., 2003; Lenders *et al*., 1995). The determination of the excretion rates of catecholamines and/or their metabolites remains the most widely used biochemical approach for initial evaluation. In this study, a highly specific HPLC procedure was used for the simultaneous determination of the 24 h urinary excretion of the catecholamines and their metabolites. In all four patients with histologically confirmed phaechromocytoma there was significant elevation of epinephrine and norepinephrine above the diagnostic cut-off values derived from the hypertensive population and an even greater elevation of fractionated metanephrine and normetanephrine indicating autonomous secretion from the tumour over the 24 h collection period. Measurement of fractionated metanephrine and normetanephrine is preferred to measurement of catecholamines because patients with phaeochromocytoma usually have larger relative increases in metanephrine and normetanephrine than catecholamines as the tumour show methylating activity (Eisenhofer *et al*., 1998). Substantial amounts of free metanephrine are formed continuously within phaeochromocytoma tumour cells and are released into the circulation independently of variations in release of the parent catecholamine (Eisenhofer *et al*., 1998) and are subsequently excreted in the urine. The 24 h urinary excretion of dopamine was elevated in all of the four histologically confirmed patients, but well below the

diagnostic cut-off point giving a sensitivity of 0% but a specificity of 96.4%. Measurement of 24 h dopamine appears to provide no additional help in diagnosing phaeochromocytoma in this study and although some authors have proposed that measuring 24 h urinary dopamine excretion may be of prognostic benefit in malignant phaeochromocytoma (Tippett *et al*., 1986; Proye *et al*., 1986), our data does not support this conclusion. Authors have also proposed that combined testing by measurements of all catecholamines as well as metanephrine and normetanephrine increases the likelihood of detecting the rare tumour that secretes only dopamine (Tippett *et al*., 1986).

The 24 h urinary excretion rate of fractionated metanephrine and normetanephrine excretion was 3-10 folds above the diagnostic cut-off value while that for 24 h urinary excretion of epinephrine and norepinephrine ranged from 3-12 folds in patients with histologically confirmed phaeochromocytoma. The interpretation of biochemical tests should be related in terms of the magnitude of the increase above the reference intervals rather than simply an increased result. A patient with significantly elevated results and relevant clinical symptoms is far likely to have a catecholamine-secreting tumour than a patient with the same symptom but a nominal, borderline increase in catecholamine or metanephrine excretion (Peaston and Weinkove, 2004).

The measurement of 24 h urinary excretion of dopamine yielded false negative results in all four patients with histologically confirmed phaeochromocytoma. The sensitivity of the 24 h urinary excretion of the catecholamines, epinephrine and norepinephrine as well as their metabolites, metanephrine and normetanephrine was 100%. Previous studies have shown that measurement of urinary metanephrines yielded false-negative results in a few patients (Bravo *et al*., 1979; Stein and Black, 1977). In addition, false-positive results are a major concern with the sensitive biochemical tests which are used in the diagnosis of phaeochromocytoma. In most studies, there is a certain percentage of false positive results observed when reference intervals are established using the 95% confidence intervals of values from a reference population. In many cases, false positive results may reflect an acute event with hyperadrenergia (Kudva *et al*., 2003). Elevated 24 h catecholamines and metanephrines above the cut-off values were observed in subjects in the hypertensive groups, although excretion rates did not overlap with values in the phaeochromocytoma group. It is suggested by Lenders *et al*. (1995) that false-positive results of the measurement of urinary metanephrine levels probably reflected a lack of treatment interruptions or dietary restrictions. False positive results may occur in patients without phaeochromocytoma who has secondary hypertension or cardiac disease rather than uncomplicated essential hypertension or patients with renal failure. The use of HPLC to simultaneously measure urinary metanephrines and catecholamines almost eliminates false positive results that are caused by interference from food or drugs (Trouvin and Billaud-Mesguich, 1987).

In this study, the 24 h urinary excretion of fractionated metanephrine had a sensitivity of 100%, negative predictive value of 100% and positive predictive value of 33.33%. These results compared favourably with that reported by Heron *et al*. (1995) where the 24 h urinary metanephrine concentrations had a sensitivity of 95%, negative predictive value of 99.9% and positive predictive value of 46.3% (Heron *et al*., 1995). However, one cannot discount the problems associated with 24 h urine collections as this is difficult in an outpatient setting and hospitalization may be required to ensure that collection is complete and that proper collection techniques are used. False negative results could result from under collection of 24 h urinary specimens with subsequently falsely low 24 h fractionated metanephrines and catecholamine measurements while false positive results of measurement of urinary metanephrine concentrations probably reflect a urine collection period that exceeded 24 h. This will either underestimate or overestimate the urinary metanephrine excretion. There are a few studies that have investigated the usefulness of metanephrine-to-creatinine ratio in establishing a biochemical diagnosis of phaeochromocytoma by normalizing the fractionated metanephrine excretion for creatinine levels with the aim of making correction for inaccurate urine collection (Heron *et al*., 1995; Sullivan and Soloman, 1975; Kaplan *et al*., 1977). In this study the

catecholamines and metanephrines concentrations were all corrected for urinary creatinine excretion. The sensitivity of the catecholamine-to-creatinine and metanephrine-to-creatinine ratios was 100%, however there was only improvement in specificity and positive predictive value for the metanephrine-to-creatinine ratio with values increasing from 92.9 to 99.1% and 33.33 to 80% respectively. There were 7 patients in the hypertensive group which had elevated urinary metanephrine concentrations but a normal urinary fractionated metanephrine-to-creatinine ratio.

Several studies have highlighted the benefits of measuring urinary fractionated metanephrines as the front-line test for discriminating secondary causes of hypertension and phaeochromocytoma (Grahmam *et al*., 1993; Hernandez *et al*., 2000). Combining measurements of 24 h urinary fractionated metanephrines and catecholamines as done in this study can be useful to distinguish borderline increases in catecholamine excretions from non-tumour sources or when clinical suspicion indicates the possible presence of a phaechromocytoma. Whilst the combined measurements may be preferable due to a lower likelihood of false positive results than with measurements of plasma free metanephrines (Eisenhofer, 2003), normal plasma metanephrine concentrations have been found to effectively rule out the diagnosis of phaeochromocytoma in asymptomatic patients (Sawka *et al*., 2003). These authors also suggest that fractionated plasma metanephrines may be the biological test of choice in high-risk patients (those with a familial syndrome or vascular adrenal) (Sawka *et al*., 2003). A problem with the use of plasma catecholamine for the diagnosis of phaeochromocytoma is that some tumours are quiescent and may not secrete large amounts of catecholamines while other tumours appear to secrete catecholamines episodically (Hernandez *et al*., 2000). Therefore a single plasma catecholamine assay may be negative if the blood is collected during the intervals between surges of high blood pressure (Plouin *et al*., 1981).

The limitation of our retrospective study is the small size of patients with histologically confirmed phaeochromocytoma which may have affected power to detect differences in sensitivities. Future research will include the quantification of urine fractionated metanephrine and normetanephrine as well as catecholamines in a larger population of patients with phaeochromocytoma using liquid chromatography-tandem mass spectrometry.

In conclusion, our results showed that measurement of 24 h urinary excretion of fractionated metanephrine and normetanephrine combined with measurement of epinephrine and norepinephrine are highly sensitive and specific biochemical indices for the detection of phaeochromocytoma. Urinary excretion of fractionated normetanephrine was the best discriminator of patients with essential hypertension and phaechromocytoma while the metanephrine-to-creatinine ratio showed better specificity and positive predictive value compared with 24 h urinary excretion of metanephrine.

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