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The role of metanephrines in the diagnosis of pheochromocytoma

Emad Rezkallah[1], Andew Elsaify[2], Wael Elsaify[2]
[1] Department of General Surgery, James Cook University Hospital, England
[2] Medical School, MISR University for Science and Technology, Egypt

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Abstract
Background: Pheochromocytomas are rare neuroendocrine catecholamine-producing tumors that may arise from the adrenal medulla (PHEO) or from extra-adrenal paraganglionic tissues (PGLs). The diagnosis of PHEO/PGLs may be challenging and depends mainly on the biochemical and radiological investigations.
Methods: Retrospective review of 26 patients who had histologically proven PHEO/PGLs in our surgical department from 2011 to 2021. These patients were compared to another set of 26 patients who had adrenalectomy for other adrenal pathologies. The plasma and 24-hour urinary levels of metanephrines (metanephrine and normetanephrine) were evaluated in both groups to detect the sensitivity and specificity of metanephrines. Additionally, these levels were correlated with the tumor size using SPSS 25 program.
Results: 57.7% of the patients (15) were females and 42.3% (11) were males. The mean age of the patients was 56.32 ± 17.38 years of age. After exclusion of the 2 hormonally silent cases, the sensitivity and specificity of the plasma metanephrines tests were about 95.8% and 98%, respectively. On the other hand, the sensitivity and specificity for 24-hour urinary metanephrines were about 83.3% and 98%, respectively. The relationship between the tumor diameter and the summed plasma metanephrines concentrations was relatively strong ($r = 0.472$, $P < 0.05$), while the relation between the tumor diameter and summed 24-hour Urinary metanephrine was potentially strong ($r = 0.354$, $P = 0.083$).
Conclusion: The test for the plasma/24-hour urinary metanephrines is now considered the best diagnostic tool for investigating PHEO/PGLs. Metanephrines levels can help to predict the tumor size and assist in decision making.

Keywords: pheochromocytoma, metanephrines, tumor size, sensitivity, specificity

Introduction

Pheochromocytomas are rare neuroendocrine catecholamine-producing tumors that arise from the chromaffin cells of either the adrenal medulla (Pheochromocytomas/ PHEO) or extra-adrenal paraganglionic tissues (paraganglioma/ PGLs) [1]. PGLs can arise from both the sympathetic and parasympathetic nervous systems; however, they preferentially arise along the sympathetic chain in the chest, abdomen and pelvis, or along the parasympathetic chain in the head and neck. It was estimated that the annual incidence of PHEO/PGLs is between two and eight per million [2] The peak incidence of PHEO/PGLs is in the third to fifth decade of life. Incidental finding of PHEO/PGLs on imaging, obtained for non-adrenal causes, accounts for about 10% to 49% of the cases [3, 4]. It was reported that 15% to 25% of PHEO/PGLs arise in extra-adrenal chromaffin tissue [5]. Studies have proven that 5% of adrenal Pheochromocytomas and 33% paragangliomas are malignant [1]. Although most of PHEO/PGLs produce, store, metabolize, and secrete catecholamines or their metabolites [2], they rarely present as biochemically silent [6]. It is considered that PHEO/PGLs are responsible for about 0.05% to 0.1% of patients with sustained hypertension. However, about 50% of PHEO/PGLs may present with paroxysmal hypertension or normotension. Symptoms may include headache, sweating and tachycardia. Despite the valuable information that can be provided by magnetic resonance imaging (MRI) or computed tomography (CT) techniques, the diagnosis of Pheochromocytoma is still based on the overproduction of catecholamines. Determinations of plasma and urinary epinephrine and norepinephrine levels are essential for diagnosis. However, these levels may be falsely negative in patients with intermittently secreting tumors or even biochemically silent Pheochromocytoma [7]. Metanephrines (normetanephrine and metanephrine) have been utilized as an alternative diagnostic tool [8, 9]. Metanephrines are O-methylated metabolites of norepinephrine and epinephrine which are produced by the enzyme catechol-O methyltransferase (COMT) and are rapidly metabolized to sulfate conjugates by the enzyme monoamine- preferring phenol sulfotransferase. The sulfate conjugates are present in plasma and urine in concentrations > 25-fold higher than the free metanephrines levels [10]. Thus, Assays of urinary metanephrines include mainly measurements of sulfate-conjugated derivatives (metanephrines metabolites) [11].
The aim of the current study is to examine whether the measurement of the plasma and 24-hour urinary metanephrines levels can help in the diagnosis of PHEO/PGLs and whether their levels correlate with the size of the tumor. This can help in the diagnosis and management of PHEO/PGLs patients.

Patients and Methods

Retrospective review of all pheochromocytoma/ paraganglioma patients who had adrenalectomy in our general surgery department during the period from 2011 to 2021. Patients' data and lab results were obtained from the hospital recording system and Web ICE. The majority of the patients presented with clinical manifestation of catecholamines excess. In a minority, no clinical

manifestations were detected apart from incidental finding of adrenal mass on imaging. All patients with adrenal mass or atypical presentation of hypertension had been assessed for catecholamine secreting-tumors. History taking and clinical examination were obtained in all patients. Hormonal analysis included plasma metanephrines (metanephrine and normetanephrine), 24h-urinary excretion of metanephrines (metanephrine and normetanephrine), plasma baseline catecholamine level, plasma cortisol level, low dose dexamethasone suppression test, 24-hour urinary free cortisol excretion, serum DHEAS, 17α-OH progesterone, serum aldosterone, plasma renin activity (in patients with hypokalaemia and hypertension). For plasma metanephrines, EDTA whole blood samples were preferred, minimum sample volume was 1 mL EDTA whole blood and minimum assay volume was 100 μL plasma. Whole blood samples were transported to the lab in ice and arrived within 2 hours of sampling. Otherwise, frozen samples could be used for at least 6 months. The routine practice in our study was to collect samples from seated patients for plasma metanephrines. Our reference ranges were based on a seated reference population:

- **Plasma normetanephrine:** < 1180 pmol/L
- **Plasma metanephrine:** < 510 pmol/L

Reference values for 24-hour urinary metanephrines were

- urinary metanephrines < 1.8 umol/24h
- urinary normetnephrines < 3 umol/24h

Plasma metanephrines values above the references were suspected for PHEO/PGLs, where values > 4 times the upper reference interval were more consistent with PHEO/PGLs. Liquid chromatography-tandem mass spectrometers were used for analysis of the samples.

Patients were advised to abstain from caffeinated and decaffeinated beverages overnight and discontinue all medications that may affect the plasma and urinary catecholamine or metanephrine concentrations prior to sampling for at least 5 days to avoid any false positive results, i.e. tricyclic antidepressants, anti-hypertensive drugs (e.g. α- and β-adrenergic receptor blockers and calcium channel blockers), monoamine oxidase inhibitors, Dopa-related drugs, and various sympathomimetic and stimulant drugs. Imaging studies included Computerized tomography (CT) and Magnetic resonance imaging (MRI) scans. Other studies like 123I-meta-iodo-benzyl-guanidine (MIBG) and positron emission tomography (PET-CT) also have been utilized.

Every individual case was discussed in an endocrine MDT meeting including a surgeon, endocrinologist, pathologist, radiologist and anesthetist. Blood pressure control preoperatively was achieved by the use of α and β antagonists. We had 26 patients with confirmed diagnosis of PHEO/PGLs. Their data were compared with the data of another set of 26 patients with other adrenal pathologies to detect the sensitivity and specificity of metanephrines measurement in the diagnosis of PHEO/PGLs. 2×2 tables have been used to calculate the sensitivity and specificity values. Histopathology results were reviewed for all patients with proven diagnosis of PHEO/PGLs to obtain the exact tumor size. Statistical analysis was carried out using SPSS version 25 to obtain the correlation coefficient between the tumor sizes and the plasma/24-hour urinary metanephrines levels.

Results

Table 1: Patients demographics and characteristics

Variable		Value
Age	Average	56.32 ± 17.38 years
	Youngest	17 years
	Oldest	82 years
Gender	Males	11 (42.3%)
	Females	15 (57.7%)
Laterality	Right	14 (53.85%)
	Left	12 (46.2%)
Genetic syndromes	No association	24 (92%)
	MEN2	1 (4%)
	SDHB	1 (4%)
Tumor Diameter	Average	4.88 ± 1.995 cm
	Smallest	1.9 cm
	Largest	8.7cm
Diagnosis	Pheochromocytoma	22 (84.6%)
	Paraganglioma	3 (11.5%)
	Composite PHEO/PGL	1 (4%)
Functional	Active	24 (92%)
	Silent	2 (8%)

26 patients with a confirmed diagnosis of pheochromocytomas/ paragangliomas were included in our study. 15 patients (57.7%) were females and 11 (42.3%) were males. The mean age was 56.32 ± 17.38 years of age. The youngest patient was 17 years old, while the oldest one was 82 years old. Two patients were diagnosed with pheochromocytoma during pregnancy. The mean tumor size was 4.88 ± 1.99 cm. The smallest tumor was 1.9 cm and the largest one was 8.7 cm. 12 patients had left sided tumor (46.2%) and 14 patients had right sided tumors (53.8%). 22 patients had histology proven pheochromocytomas, one patient had combined PHEO/PGLs and three patients got paragangliomas; two of them were hormonally silent. (Figure 5) (Table 1) After exclusion of the 2 hormonally silent cases, the sensitivity and specificity of the plasma metanephrines tests were about 95.8% and 98%, respectively. On the other hand, the sensitivity and specificity for 24-hour urinary metanephrines were about 83.3% and 98%, respectively. The mean level of plasma normetanerphrines was 5443.9 ± 5182.48 pmol/L, while for plasma metanephrine it was 2357 ± 2457.7 pmol/L. The mean level of 24-hour urinary normetanerphrines 18.995 ± 41.61 umol/24h, while for 24-hour urinary metanerphrines was 7.22 ± 5.37 umol/24h. Aggressive pheochromocytoma features were detected only in one patient. Genetic associated syndromes were found in two patients; one had MEN2 syndrome and one had Succinate dehydrogenase subunit B (SDHB) gene mutations. No recurrence was detected in our patients. We found a strong positive relationship (r = 0.472, P < 0.05) between the tumor diameter and the summed plasma concentrations of free normetanephrine and metanephrine. The relation between the tumor diameter and free plasma concentrations of normetanephrine was also relatively strong (r = 0.447, P < 0.05), while the relation between the tumor size and the free plasma level of metanephrine was relatively less strong (r = 0. 237, P = 0.244). The relation between the tumor diameter and summed 24-hour urinary metanephrine was potentially strong (r = 0.354, P = 0. 083). (Figures 1- 4) (Table 2)

Table 2: Statistical results

	Plasma metanephrine	24-hour urinary metanephrine
Sensitivity	95.8%	83.3%
Specificity	98%	98%
Correlation with tumor size	r = 0.472, P < 0.05	r = 0. 354, P ~ 0. 083

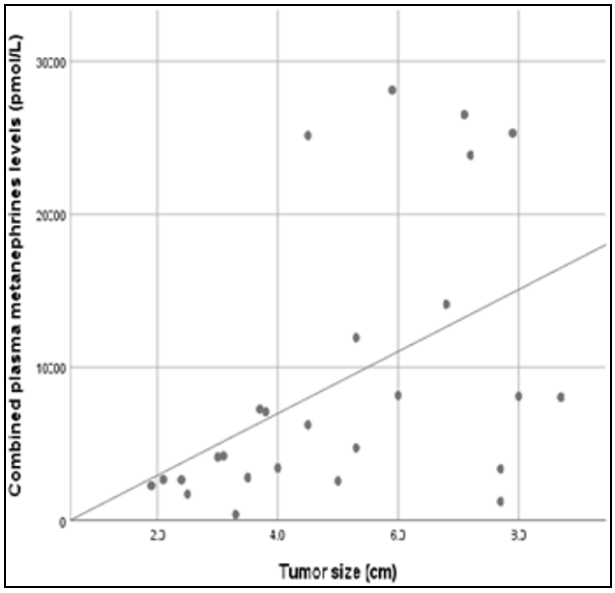

Fig 1: Simple Scatter with Fit Line of combined plasma metanephrines by tumor size

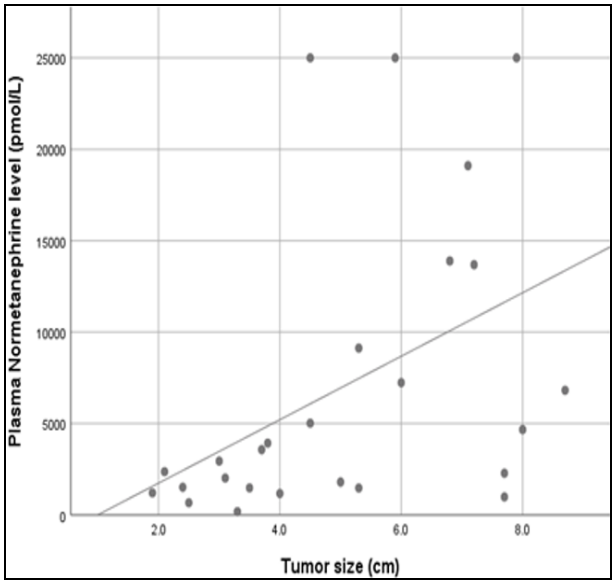

Fig 2: Simple Scatter with Fit Line of free plasma normetanephrines by tumor size

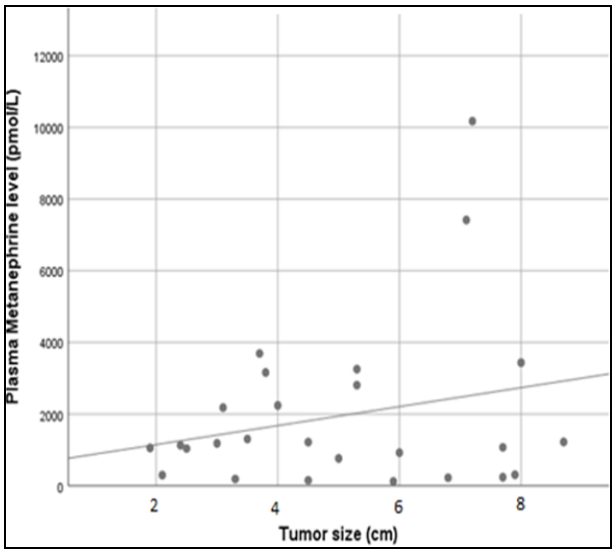

Fig 3: Simple Scatter with Fit Line of free plasma metanephrines by tumor size

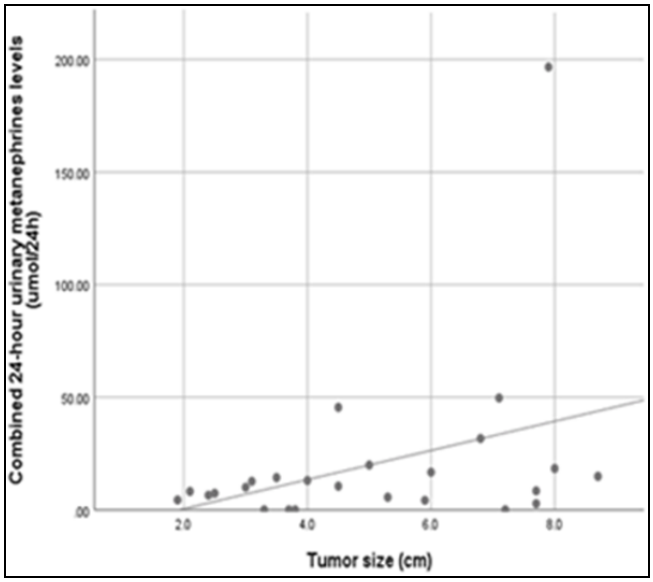

Fig 4: Simple Scatter with Fit Line of combined 24-hour urinary metanephrines by tumor size

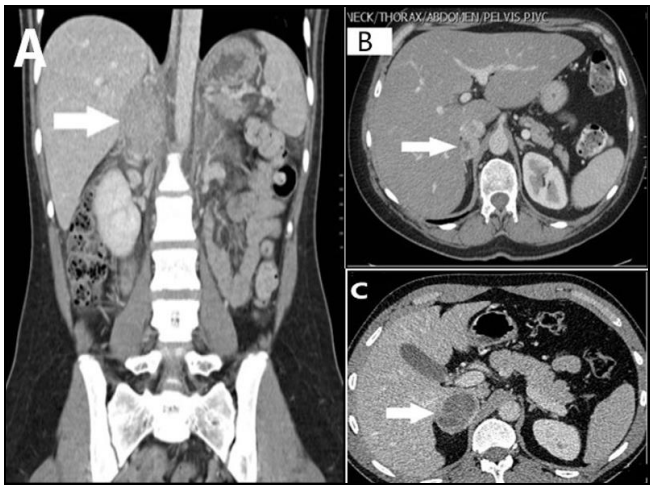

Fig 5: MSCT abdomen showing (A) an ill-defined enlargement of the right adrenal measuring about 4.5 cm, (B) a well circumscribed, rounded right adrenal nodule, with peripheral enhancement and centrally low density measuring about 3.5 x 2.3 cm, (c) showing a well-circumscribed mass arising within the medial limb of the right adrenal gland measuring 5.3 x 3.8 cm in maximal axial dimensions.

Discussion

Pheochromocytoma is a rare tumor that arises from the chromaffin cells in adrenal medulla or other paraganglia in the body [1]. Most of pheochromocytomas are sporadic and it was believed in the past that nearly 10% of PHEO/PGLs may be familial. However, recent studies reported that up to 24% of the cases are familial where they may be associated with certain genetic syndromes like multiple endocrine neoplasia (2A and 2B), Succinate dehydrogenase subunit B (SDHB) gene mutations, Von-Hippel-Lindau syndrome, cerebellar hemangio-blastoma, Sturge-Weber syndrome, and tuberous sclerosis [12]. Two of our patients had genetic mutation; one had MEN2 and one had SDHB. There was no significant predominance between males and females (11 vs. 15) in our study; however other studies reported slight predominance of male patients [13]. The average age for diagnosis in our study was 56.32 ± 17.38 years. Other studies reported mean age of 47.1 years for diagnosis [14]. Three cases in the current study had paragangliomas and

one case had composite pheochromocytoma and paragangliomas with each component constituted approximately 50% of the tumor. Other studies reported that 75-85% of pheochromocytomas are adrenal and remaining 15-25% are extra-adrenal paragangliomas [5]. There was no major difference regarding the side of the tumor (right: 14, left: 12).

Pheochromocytoma is responsible for about 0.01-0.1% of the cases of hypertension [15]. A patient of hypertension should be investigated for pheochromocytoma if he/she has atypical clinical picture such as severe hypertension, hypertensive crisis, resistant hypertension, age of presentation < 20 years or > 50 years, family history of pheochromocytoma-associated hereditary syndromes or proven adrenal mass on imaging [16]. The classical triad of pheochromocytoma includes episodic headache, sweating, and hypertension. However, it is estimated that about 50% of PHEO/PGLs may present with paroxysmal hypertension or even normotension. It is worth mentioning that unrecognized pheochromocytomas may cause death as a result of a hypertensive crisis, arrhythmia, myocardial infarction, or multisystem crisis [17].

The detection and localization of pheochromocytoma is potentially challenging, which have been currently facilitated by the recent advances in biochemistry and radiology. Computerized tomography (CT) and Magnetic resonance imaging (MRI) scans can have about 95% sensitivity and 70% specificity for adrenal Pheochromocytomas. On T1 imaging pheochromocytomas are isointense to liver, kidney, and muscle while highly intense signal is seen on T2 images and no signal loss on opposed phase images because of the absence of fat in Pheochromocytomas [18, 19]. Other advanced imaging modalities that can be utilized in the diagnosis of PHEO/PGLs may include 123I-meta-iodo-benzyl-guanidine (MIBG), 18-fluoro-dihydroxyphenylalanine (18F-DOPA) positron emission tomography (PET-CT), 18-fluorodeoxyglucose (18F-FDG) PET-CT.

It was found that the secretion of catecholamines is episodic; thus single estimation of urinary or plasma epinephrine and norepinephrine is likely to miss the diagnosis of pheochromocytoma in many cases, especially in familial cases which may have false negative results in up to 29% of the cases [20]. Nevertheless, the process of catecholamine metabolism inside the pheochromocytomas is constant [21]. Consequently, assessment of metabolites of epinephrine and norepinephrine; metanephrine and normetanephrine respectively, is currently considered the best screening test for pheochromocytoma. In the current study, we reported high sensitivity and specificity rates for the plasma metanephrines test; 95.8% and 98%, respectively. Other studies reported similar high (97-100%) sensitivity, but lower specificity (82-85%) [22]. Moreover, the sensitivity and specificity rates for 24-hour urinary metanephrines and normetanephrines were about 83.3% and 98%, respectively. G Eisenhofer *et al*. reported higher sensitivity (97%) but similar specificity (98%) rates [22]. We had low false positive rates in our study which may be attributed to following the standards in collecting blood samples, where the patients were initially instructed to avoid any medication that may affect the test results for at least five days, mainly tricyclic antidepressants, anti-hypertensive drugs (e.g. α- and β-adrenergic receptor blockers and calcium channel blockers), monoamine oxidase inhibitors, Dopa-related drugs, and various sympathomimetic and stimulant drugs. Patients were advised to refrain from nicotine and alcohol for at least 12 hours and to fast overnight before blood sampling. The protocol in our study was to collect the blood sample from the patient while in seated position.

Many studies well documented no or poor relationships of pheochromocytoma tumor mass with the urinary or plasma concentrations of catecholamines despite reporting strong relationships with the catecholamine metabolites (metanephrine and normetanephrine) [20, 23, 24]. These differences reflect the variable and intermittent secretion of catecholamines by the tumor, compared with the continuous production of free metanephrines within tumor cells by the enzyme, catechol-O methyltransferase (COMT), this process that is independent of the catecholamine release [25].

In the current study, we concluded a strong positive relationship ($r = 0.472$, $P < 0.05$) between the tumor diameter and the summed plasma concentrations of free normetanephrine and metanephrine. In addition, the relation between the tumor diameter and summed 24-hour urinary metanephrine was also potentially strong ($r = 0.354$, $P = 0.083$).

Indeed, the high sensitivity and specificity of plasma and urinary metanephrines and the strong relations between these levels and the tumor size have many implementations in pheochromocytoma management. It would not only help in the diagnosis of PHEO/PGLs, but also can help to predict the tumor size. Additionally, it would help to predict the difficulties in controlling the blood pressure of patients with very high levels of plasma/24-hour metanephrines with consequently large volume tumors. Moreover, it would help to avoid unnecessary costly tests and imaging procedures.

Conclusion

Pheochromocytomas and paragangliomas are rare catecholamine-secreting neuro-endocrine tumors. Plasma and 24-hour urinary metanephrines are of great value in the diagnosis of these tumors as they are continuously produced by the tumor cells in contrast to catecholamines that may be intermittently produced. There are strong relations between the size of these tumors and the plasma /urinary concentration of these metabolites, which can help to predict the size of the tumor even before imaging. Proper implication of the information obtained from measuring the levels of metanephrines in plasma and urine together with the results of high quality imaging techniques can be of great value in diagnosis and decision-making regarding PHEO/PGLs patients.

Ethical approval

Not applicable.

Sources of funding

None.

Conflicts of interest

None.

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