PREDICTIVE VALUE OF CHROMOGRANIN A IN A DIAGNOSIS TOWARDS PHEOCHROMOCYTOMA IN ADRENAL INCIDENTALOMA

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Abstract

Context. Some adrenal tumors, such as pheochromocytoma, can be life-threatening. Therefore it is crucial to distinguish them from other lesions, especially prior to surgery. Chromogranin A (CgA) seems to potentially be a good marker for tumors of chromaffin origin.

Objective. To assess the differentiating value of CgA in the diagnostic work-up of pheochromocytoma.

Design. Retrospective study of operated patients with adrenal incidentaloma with lesions > 10 Hounsfield's units (HU) on CT.

Subjects and Methods. Thirty patients (11 males, 19 females; aged 61.5±21 years) were enrolled in the study. Patients using medications interfering with the assessment of CgA and metanephrines were excluded. Two groups were formed: those with pheochromocytoma (Ph, n=16) and those with non-pheochromocytoma (N-Ph, n=14) lesions. Data included radiological features of masses, serum CgA and 24-hour urine metanephrines (24 - HUM) concentrations.

Results. No difference in 24-HUM level nor tumor size or density was found between groups Ph and N-Ph. Median serum CgA concentration was higher in Ph group compared to the N-Ph: 99.35 (68.12-172.73) vs. 52.92 (34.37-101.26) ng/mL, respectively (P=0.04). In Ph group, the size of the lesion correlated negatively with density ($r = -0.53$, $P = 0.042$). No significant correlation in CgA, 24-HUM, density or size of the lesion was found. Performed curve receiver operating characteristic (ROC) showed AUC=0.7232 for CgA. Taking into account CgA serum value of ≤ 50 ng/mL (sensitivity: 93.75%, specificity: 50.00%, $P = 0.012$), we proposed an algorithm for management of lesions > 10 HU on CT.

Conclusion. CgA level ≤ 50 ng/mL might be useful in initial screening evidence for the exclusion of pheochromocytoma. It is crucial to eliminate factors interfering with the measurements.

Key words: pheochromocytoma, adrenal incidentaloma, chromogranin A, chromaffin cells.

INTRODUCTION

Due to the present intensive development of imaging techniques, adrenal tumors are being diagnosed more frequently (1). The majority are clinically silent, but some, like pheochromocytoma, may be life-threatening (2). Therefore it is crucial to distinguish chromaffin cell tumors from other lesions, and to remove functioning and malignant lesions without performing an adrenalectomy in non-functioning, benign tumors (3, 4). The diagnosis of adrenal mass is based on computed tomography (CT), clinical picture and biochemical tests. However, none of these methods represent optimal diagnostic accuracy. CT plays a significant role in detection of adrenal incidentaloma (AI). The latter term refers to each tumor > 1 cm of the adrenal gland diagnosed incidentally during chest or abdomen imaging procedures (5). An adrenal tumor with a density > 10 Hounsfield Units (HU) may represent a lipid poor adenoma, pheochromocytoma, metastasis or adrenocortical carcinoma. As a CT picture of pheochromocytoma can present a wide range of features imitating other lesions, some authors describe them as imaging "chameleons" (6). Surgical treatment should be offered in case of hormonal activity of an adrenal lesion or a suspicious CT picture. The clinical picture of pheochromocytoma can be confusing, varying between asymptomatic cases, nonspecific and severe symptoms. Pheochromocytoma is a rare type of tumor, mostly benign, isolated (more frequently) or familial, in which case it is correlated with Von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type: IIA and IIB (MENIIA, MENIIB) or neurofibromatosis type 1 (NF-1). The symptoms (an increase in blood pressure, headache, diaphoresis, tachycardia, anxiety and pallor) are a result of excessive secretion of catecholamines (7, 8). Biochemical tests have limitations; therefore applied methods, interpretation of results, as well as

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sources of interference should always be taken into further consideration. The recommended biochemical tests for pheochromocytoma diagnosis representing the highest specificity and sensitivity include urinary or plasma fractionated metanephrines by liquid chromatography-mass spectrometry method (LCMS/MS), radioimmunoassay (RIA) and enzyme immunoassay (EIA) (9-12). Nevertheless, many centers use other, less accurate but more accessible methods, based on determining 24-hour urine metanephrines (24-HUM) (9, 11, 12). These may give both falsely positive and falsely negative results. In any case, patients with pheochromocytoma, even if clinically or biochemically silent, require surgery and proper preoperative work-up (12, 13). In this study we looked for biochemical factors, besides metanephrines measurement, which could help to exclude pheochromocytoma. Chromogranin A (CgA) is a secretory glycoprotein stored in chromaffin cells, which makes it a potentially good marker for tumors of chromaffin origin (14, 15). We aimed to find the cut-off level of CgA concentration that could differentiate pheochromocytoma from other tumors with the density > 10 HU.

PATIENTS AND METHODS

Subjects

Thirty-seven consecutive patients (mean age: 54 ± 15 years, 23 females and 14 males) admitted to the hospital between the years 2010-2015 with adrenal lesions of > 10 HU assessed on CT scan by radiologist (research of interest places, ROI) who underwent surgery, were qualified to the study.

Exclusion criteria

Patients on medication interfering with results of either CgA or 24-HUM, which included mainly: proton pump inhibitors, H₂-blockers, glucocorticoids – for CgA and paracetamol, monoamine oxidase inhibitors, tricyclic antidepressants, levodopa, anxiolytics, antihypertensives (as labetalol and sotalol), stimulants (e.g. caffeine, nicotine), sympathomimetics (e.g. amphetamines, ephedrine) (16) – for 24-HUM were excluded from the further key analysis.

Study design

We have analyzed the data of thirty patients; mean age: 61.5 ± 21 years; 19 females and 11 males; 7 patients were excluded because of taking interfering medications. Based on histopathological examination, patients were divided into two groups: those with

pheochromocytoma (Ph, n=16) and those with non-pheochromocytoma (N-Ph, n=14) lesions. We have analyzed radiological features of lesions (size and density), coexistence of other neoplasms, age, sex, serum CgA and 24-HUM concentration. Based on the results, we conducted a receiver operating characteristic (ROC) curve for CgA, determined appropriate cut-off values, as well as proposed an algorithm for exclusion-screening for pheochromocytoma in case of detected incidentaloma > 10 HU on CT.

Moreover, we have conducted an additional explorative analysis including all patients primarily enrolled to the study (n=37, Ph=17, NPh=20), aiming to compare CgA level in the group of patients taking interfering drugs (n=7) with drugs-free group (n=30).

Assays

The blood samples were prepared in a fasted state and serum was adequately separated and stored at -20°C until analysis. The serum CgA level was assessed by enzyme-linked immunosorbent assay (Cisbio Bioassays, Massachusetts, United States). 24-HUM concentration was measured by spectrophotometric assay (Laboratory of Clinical Biochemistry, National Heart Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Bethesda, Md.U.S.A.) (17).

Statistical analysis

The SAS 9.3 was used for a statistical analysis. Data were expressed as either means $\pm$ standard deviation (SD) or median and quartiles (brackets), when appropriate. We used Spearman's rank test for correlations of ranked, quantitative variables in both groups. Wilcoxon's two-sided test and Fisher's exact test were used to identify differences between groups. A statistical significance value P was set at <0.05.

RESULTS

The demographic characteristics of the groups are presented in Figure 1.

Figure 1. The demographic characteristics of the patients.

Figure 2. Serum CgA concentrations in studied groups, Wilcoxon's two-sided test, $P = 0.04$.

Abbreviations: CgA – chromogranin A, N-Ph – non-pheochromocytoma, Ph – pheochromocytoma.

Figure 4. The proposed algorithm for patients' with adrenal lesion >10 HU in CT scan.

Abbreviations: CgA – chromogranin A, CT – computed tomography, HU – Hounsfield's units.

Median serum CgA concentration was higher in Ph group compared to the N-Ph: 99.35 (68.12-172.73) vs. 52.92 (34.37-101.26) ng/mL, respectively ($P=0.04$) (Fig. 2). Performed ROC showed area under the curve (AUC), that equaled 0.7232 for CgA (Fig. 3).

Positive results for CgA made us search for a "cut-off" diagnostic and discriminative value.

Considering the routinely presumptive diagnostic value ($90 \text{ ng/mL} \pm 40 \text{ ng/mL}$) - we noted 50 ng/mL and 60 ng/mL as having the highest sensitivity and specificity (Table 2). Whereas values of positive predictive value (PPV) varied between 68-78% for all cut-offs, negative predictive value (NPV) was significantly higher in case of 50 ng/mL (87%) compared to other values.

Taking into account CgA serum value of ≤ 50

Figure 3. Plot showing ROC applied to CgA. The AUC equaled 0.7232.

Abbreviations: AUC – area under the curve, CgA – chromogranin A, ROC – receiver operating characteristic curve.

ng/mL (sensitivity: 93.75%, specificity: 50%, $P=0.012$), we proposed an algorithm for the management of lesions > 10 HU on CT (Fig. 4).

Although our research emphasizes the discriminating role of CgA, we propose to perform 24-HUM measurement first according to the current recommendations (11, 12).

24-HUM was measured in both groups. It is noteworthy that there was no difference in 24-HUM level, tumor size or density between Ph and N-Ph groups (Table 1). Patients' age and sex were also not different, taking into account 24-HUM level of $\leq 700 \mu\text{g}/24\text{h}$ as an addition to CgA measurement, characterized with sensitivity and specificity 100% and 60%, respectively ($P=0.046$).

In the Ph group, the size of a lesion was negatively and significantly correlated with density of a lesion ($r=-0.53$, $P=0.042$). Besides CgA, 24-HUM, density and size of a lesion were not significantly correlated with one another in either studied group.

N-Ph group consisted of lipid-poor adrenal adenomas ($n=9$), adrenocortical carcinomas ($n=2$), epithelioid angiosarcoma ($n=1$), adrenal hyperplasia ($n=1$), metastasis to adrenal gland ($n=1$). As for Ph group, we noticed three cases of rare syndromes: MENIIA, NF-1, VHL.

We performed additional, explorative analyses to further study and include patients taking interfering drugs. These showed a lack of statistically significant difference between Ph and NPh groups in terms of CgA concentration – 100.32 (71.72-178.1) vs. 74.84 (44.51-136.13) ng/mL ($P=0.17$). Independently of lesion etiology, analysis of CgA concentration in patients

Table 1. Empirically found CgA concentrations – “cut-off”, presenting respective sensitivity and specificity values in detecting adrenal lesion of >10 HU in CT scan

CgA “cut-off” value [ng/mL]	N	Ph-p	Ph-n	Sens (%)	Spec (%)	Fp (%)	Fn (%)	PPV (%)	NPV (%)
50	8	1	7	93.75	50.00	50.00	6.25	68.18	87.50
60	10	2	8	87.50	57.14	42.86	12.50	70.00	80.00
70	13	4	9	75.00	64.29	35.01	25.00	70.59	69.23
80	15	6	9	62.50	64.29	35.01	37.50	66.67	60.00
90	16	7	9	56.25	64.29	35.01	43.75	64.29	56.25
100	18	8	10	50.00	71.43	28.57	50.00	66.67	55.56
110	20	9	11	43.75	78.57	21.43	56.25	70.00	55.00
120	21	9	12	43.75	85.71	14.29	56.25	77.78	57.14

Abbreviations: CgA – chromogranin A, Fn – false negative, Fp – false positive, N – number of patients, NPV – negative predictive value, Ph-n – pheochromocytoma negative, Ph-p – pheochromocytoma positive, PPV – positive predictive value, Sens – sensitivity, Spec – specificity.

Figure 5. A selection of radiological pictures (CT scans) with descriptions, presenting a variety of adrenal masses in studied group.

A, B, C: Right adrenal gland: inhomogeneous (2-13HU), round-shaped lesion with clear margins, 31mm in diameter. Not a typical picture of a benign adrenal adenoma, may indicate lipid poor adenoma. Histopathology: adrenal adenoma. **D:** Right adrenal gland: homogeneous, well-defined, oval-shaped mass, 29x14mm, 0-6 HU; picture diagnostic of a benign adrenal adenoma. Histopathology: adrenal adenoma. **E, F:** Left adrenal gland: adrenal mass with irregular margins, 59x42mm, containing calcifications and areas of fat (-60HU). The image is suggestive of myelolipoma or atypical adrenal adenoma, but due to presence of calcifications an adrenal cancer cannot be excluded. Histopathology: adrenal hypertrophy. **G:** Right adrenal gland: inhomogeneous, well-defined, round-shaped mass, 12mm in diameter, 30HU. Not a typical picture of a benign adrenal adenoma, needs a discrimination between pheochromocytoma, adrenal cancer and metastasis. Histopathology: pheochromocytoma. **H:** Right adrenal gland: inhomogeneous, round-shaped lesion with clear margins, 28mm in diameter, 48HU. Not a typical picture of a benign adrenal adenoma, needs a discrimination between pheochromocytoma, adrenal cancer and metastasis. Histopathology: pheochromocytoma.

Table 2. A comparison of urine metanephrines, tumor size and density

Variable	Ph	N-Ph	P
24-HUM [ug/24h]	1213.00 (625.00-1728.00)	650.00 (500.00-1140.00)	0.25
Size of lesion [mm]	31.00 (16.00-49.00)	29.00 (15.00-40.00)	0.85
Density of lesion [HU]	32.00 (11.00-34.50)	15.00 (11.00-30.00)	0.19

Abbreviations: 24-HUM – 24-hour urine metanephrines, N-Ph – non-pheochromocytoma, Ph – pheochromocytoma, HU – Hounsfield's units.

taking drugs causing false positive results (n=7) and the group of drugs-free individuals (n=30), showed significantly higher serum CgA levels in drugs-positive group vs. drugs-free individuals: 213.4 (98.61-302.56) vs. 78.41 (47.22-147.9) ng/mL, respectively (P=0.038).

DISCUSSION

When an adrenal tumors is incidentally detected, the main aim is to exclude a hormonally active

tumor, including pheochromocytoma and malignancy. Resection should be offered in case of hormonal activity (18) and suspicion of malignancy. Additionally, most non-secreting lesions larger than 6 cm should be removed due to the increased risk of malignancy, regardless of their imaging status (3).

Our study showed that CgA accompanying urine metanephrines measurement may help in excluding pheochromocytoma from other incidentally found adrenal lesions with density >10 HU. Interestingly, no

difference was found in 24-HUM between patients with or without pheochromocytoma.

The routine biochemical methods for the diagnosis of pheochromocytoma include measurement of 24-hour urine metanephrines (9, 11, 12). In our study 24-HUM concentration was measured by spectrophotometric assay. This method does not allow separate measurements of normetanephrine and metanephrine, but only total metanephrines. Separate measurements would help ensure that small or mild increases in one metabolite are not masked by normal levels of the other. Another issue is analytical interference by drugs or dietary constituents (19). In our analysis we have, however, excluded patients on medications that could potentially interfere with the 24-HUM measurement.

CgA is a non-specific biochemical marker widely used in gastro-entero-pancreatic neuroendocrine neoplasms (GEP-NENs) diagnostic workup (20). Many authors claimed the usefulness of CgA also in pheochromocytoma diagnosis (14, 21).

In our study, CgA concentration in both groups was much lower than in the majority of previous publications (22-25). According to Polish diagnostic guidelines, the obtained value of 50 ng/mL is considered to remain within the normal range (26). In one study, median levels of CgA in pheochromocytoma compared to silent adrenal incidentalomas equaled 660 (266-1478) vs. 68 (20-167) ng/mL (27). Our results were similar as described by Giovanella *et al.* which claimed that negative CgA in patients affected by incidentally discovered masses >20 mm can safely reassure clinicians about the absence of chromaffin tissue (28). In that study, a significantly higher serum CgA level was seen in patients with pheochromocytoma compared to other adrenal lesions (median levels 395 vs. 44 ng/mL, respectively). Giovanella *et al.* also showed that, compared to urinary catecholamines and their metabolites, CgA was the single most effective marker in the detection and exclusion of pheochromocytoma among patients with asymptomatic adrenal incidentaloma of > 20 mm (29). In our study we suggest the cut-off value of serum CgA ≤ 50 ng/mL as a useful tool in pheochromocytoma exclusion in the differential diagnosis of adrenal incidentaloma (sensitivity: 93.50% and specificity: 50%). Grossrubatscher *et al.* showed that either CgA combined with urine catecholamines or metanephrines exhibited sensitivity up to 100% in pheochromocytoma diagnosis compared to adrenal incidentaloma (21). In our study 24-HUM with a cut-off value of ≤ 700 $\mu\text{g/L}$, combined with serum CgA ≤ 50 ng/mL increased sensitivity and

specificity values up to 100% and 60%, respectively. It is important that the cut-off level for 24-HUM is also much lower than the value routinely applied as elevated (26, 30). Additionally, authors showed a positive correlation between tumor size and mean plasma CgA and urine metanephrine concentrations, which was not observed in our results (including serum, not plasma CgA level) (21).

As CgA concentration may be associated with other factors, e.g. heart or kidney failure, atrophic gastritis, drugs (31, 32), we have selected patients not exposed to these factors. Besides this, we have also performed an explorative analysis in patients taking interfering medications to show the association between drugs (commonly PPIs and H2-blockers) and an elevated concentration of CgA, consequently proving the pertinence of patient selection.

Typically, the majority of adenomas (70-80%) present as tumors of ≤ 10 HU (33), which are called “lipid-rich” adenomas. In our patients the relation between density and the histopathological diagnosis has not always occurred, as in cases of radiologically suspected adrenal cancer or myelolipoma (Fig. 5), which were diagnosed by histopathological study as adrenal hyperplasia (34-38). The N-Ph group from our study composed of 9 adenomas, all with density >10 HU (11-53), which indicates them as “lipid poor” adenomas (39). Therefore, the management of uncertain adrenal lesions should involve a complex of consecutive procedures, including imaging, biochemical, and immunohistochemical investigation.

In conclusion, CgA measurement accompanying urine metanephrines seems to be an effective marker helping to exclude pheochromocytoma from other adrenal lesions incidentally found on CT scan, presenting with density >10 HU. It is crucial to eliminate factors interfering with the measurements.

Conflict of interest

The authors declare that there is no conflict of interest.

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