Inhibin alpha-subunit, Melan A and MNF116 in pheochromocytomas

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Abstract

Aim: The aim of this study was to make immunohistochemical analyses with Inhibin alpha-subunit, Melan A and MNF116 (pan-Cytokeratin antibody) in pheochromocytomas, because immunohistochemistry is useful for the distinction between adrenal tumors.

Patients and Methods: We used 20 patients with pheochromocytomas submitted to laparoscopic (n=19) or classical (n=1) surgery and we have explored immunostaining with Inhibin alpha-subunit, Melan A and MNF116 in these tumors. This can be helpful when we cannot make the distinction between adrenal tumors.

Results: Pheochromocytomas did not stain with Inhibin alpha-subunit, Melan A and MNF116.

Conclusions: In our study, Inhibin alpha-subunit, Melan A and MNF116 were not sensitive for pheochromocytomas.

Keywords: pheochromocytomas, immunohistochemistry, Inhibin alpha-subunit, Melan A, MNF116.

Introduction

Pheochromocytoma is a neuroendocrine tumor of the medulla of the adrenal glands that secretes high amounts of catecholamines. Recently, with the progress of imaging techniques pheochromocytomas can be detected earlier and frequently with smaller sizes. Sometimes, it is difficult to make the distinction between adrenal tumors by pathology, imagistic appearance or hormonal evaluation and immunohistochemistry is frequently used in the differential diagnosis of adrenal neoplasms, since molecular techniques are mostly limited to research laboratories [1]. The catecholamine-synthesizing cells typically exhibit positivity for a variety of generic neuroendocrine markers and are variably positive for certain peptide hormones. Synaptophysin is present in 100% of cases, whereas chromogranin A is expressed in more than 95% [2, 3]. Generally, chromogranin immunoreactivity is more intense in normal than in neoplastic cells of paragangliotic tissue. NSE (neuron-specific enolase) is present in virtually all pheochromocytomas and paragangliomas [4]. In addition to catecholamines, serotonin immunoreactivity has been demonstrated in approximately 80% of pheochromocytomas [5]. Both pheochromocytomas and extra-adrenal paragangliomas may also contain peptide hormones including neuropeptide Y (64%), substance P (36%), calcitonin (21%), and leu- and met-enkephalin (70%) [6, 7].

Inhibin alpha was studied in adrenal tumors and although it is considered a specific marker for cortical neoplasms, Pelkey et al. reported immunoreactivity in two of 19 pheochromocytomas [8].

The presence of cytokeratin immunoreactivity in pheochromocytomas has been controversial. Kimura et al. reported cytokeratin immunoreactivity in almost 30% of pheochromocytomas using a broad-spectrum cytokeratin antibody [9]. Chetty et al. reported no immunoreactivity with cytokeratin in pheochromocytomas [10]. Another immunohistochemical marker for pheochromocytoma is CD56 (cluster of differentiation). In the normal adrenal gland, CD56 is present in the medulla and the zona glomerulosa. Pheochromocytomas are typically strongly positive for CD56 [11]. Also, there is a strong membranous markup for CD44 in pheochromocytoma [12, 13].

In pheochromocytomas, Ki-67 antigen is described as an excellent marker of the proliferative activity and a Ki-67 index (>3%) is a useful marker for distinguishing benign from malignant tumors or for predicting the malignant potential of pheochromocytomas [14].

The aim of our study was to examine immunoreactivity with Inhibin alpha-subunit, Melan A known as markers of adrenocortical tissue and MNF116 known as marker of epithelial tissue in 20 pheochromocytomas, in order to extend the knowledge of these markers in pheochromocytomas. This can be helpful when we cannot make the distinction between adrenal tumors by the regular methods.

Materials and Methods

We retrieved samples of pheochromocytomas from the pathology files of the “Floreasca” Emergency Clinical Hospital.
Hospital, Bucharest, Romania. Cases were selected in which the diagnosis was well established based on the histological features of the tumor and the history provided on the pathology report. There have been 20 pheochromocytomas, which had been diagnosed between 2005–2012 in “Constantin I. Parhon” National Institute of Endocrinology, Bucharest. The patients have presented for endocrinological evaluation of an adrenal tumor, which had been found on a computerized tomography (CT) performed because of symptoms unrelated with the adrenal tumor but not for the staging of a known malignancy (12 patients) or for endocrinological evaluation of high blood pressure (HBP) multi-drugs resistant (eight patients). All patients had adrenal CT and hormonal evaluation: plasma and urinary metanephrine, low-dose Dexamethasone suppression test (1 mg Dexamethasone overnight), plasma and urinary MN (metanephrines) and NMN (normetanephrines) and Aldosterone/Renin ratio for patients with HBP. Plasma metanephrines were measured using commercial ELISA kits (normal range for plasma Normetanephrine is 15–180 pg/mL and for Metanephrine 10–90 pg/mL). Urinary metanephrines were determined by high-performance liquid chromatography (HPLC) analysis (normal range for urinary Normetanephrine is 100–600 μg/day while for urinary Metanephrine is 50–350 μg/day).

Surgery had been performed for all these pheochromocytomas in “Floreasca” Emergency Clinical Hospital Bucharest, in the same period. We reviewed Hematoxylin–Eosin stained slides and selected routinely processed, formalin-fixed paraffin blocks containing well-preserved tumor. Immunostaining was performed (“Victor Babes” National Institute, Bucharest) using three types of antibodies for each tumor sample: Inhibin alpha (Novoceastra) clone AMY82, Melan A/MART1 (Dako) clone A103, MNF116. Neither of antibodies (we used monoclonal not polyclonal antibodies).

Statistical analysis was made using statistic functions from Microsoft Office Excel 2003 (AVERAGE, STDEV, TTEST with a p-value <0.05 being considered with statistical significance).

The Institutional Ethics Committee approved the study protocol and informed consent. All subjects provided written informed consent before participating in this study.

**Results**

We included 20 patients with pheochromocytomas: 15 women and five men. The mean age at the diagnosis was 53.09±16.625 years (51.76±17.25 years for women and 54.4±16.1 years for men). All tumors were unilateral: eight left pheochromocytomas and 12 right pheochromocytomas. Mean tumor size was 5.5±5.33±2.73/2.65 cm for left pheochromocytomas and 7.14/6.33±2.34/2.33 cm for right pheochromocytomas. The smallest tumor size was 2/2.1 cm and the biggest tumor size was 10/8 cm. The majority of tumors had >5 cm. Distribution of cases function of tumor size are shown in Table 1. Plasma and urinary MN and NMN are shown in Table 2 and it is not correlated with tumor size (p=0.023).

Preoperative treatment with Carvedilol was necessary in all cases for 14–21 days. Twelve patients had HBP and eight had normal BP; HBP was still present in eight patients after surgery.

### Table 1 – Distribution of cases function of tumor size

<table>
<thead>
<tr>
<th>Tumor size [cm]</th>
<th>No. of cases</th>
</tr>
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<tbody>
<tr>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>2–5</td>
<td>4</td>
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<tr>
<td>&gt;5</td>
<td>15</td>
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</table>

### Table 2 – Median value of plasma and urinary MN and NMN function of tumor size

<table>
<thead>
<tr>
<th>Tumor size [cm]</th>
<th>Plasma MN (μg/24 h) (N: 10–90)</th>
<th>Plasma NMN (μg/24 h) (N: 15–180)</th>
<th>Urinary MN (μg/24 h) (N: 100–600)</th>
<th>Urinary NMN (μg/24 h) (N: 50–130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5</td>
<td>111±12</td>
<td>243±19</td>
<td>721±23</td>
<td>545±14</td>
</tr>
<tr>
<td>&gt;5</td>
<td>109±17</td>
<td>251±21</td>
<td>788±31</td>
<td>601±29</td>
</tr>
</tbody>
</table>

MN – Metanephrines; NMN – Normetanephrines; N – Normal values.

All patients had laparoscopic adrenalectomy. Just in one case, conversion to open surgery was necessary because of the big tumor size (tumor size was 10 cm).

All the histopathological blocks were analyzed by immunohistochemistry using the above-mentioned antibodies: Inhibin alpha, Melan A, MNF116. Neither of the pheochromocytoma stained with either of the used antibodies (Table 3).

### Table 3 – Immunoreactivity of pheochromocytomas

<table>
<thead>
<tr>
<th>Pheochromocytoma</th>
<th>Inhibin alpha</th>
<th>Melan A</th>
<th>MNF116</th>
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In Figure 1, we can see Inhibin alpha positive immunohistochemistry and in Figure 2 Melan A positive immunohistochemistry in adjacent cortico-adrenal brown stained tissue.

**Discussion**

Inhibins are heterodimeric glycoproteins consisting of an α-subunit and either a βA- or a βB-subunit but activins are composed of β-subunits only. The main sources of the circulating Inhibin are the gonads (granulosa cells for the ovary and Sertoli cells for the testes) but the expression of the Inhibin alpha has also been found in adrenal glands and other organs: pituitary gland, liver, placenta. Immunohistochemically, Inhibin/Activin β-subunit is strongly positive in the normal adrenal medulla, but the cortex was negative. Weak immunohistochemical positivity for Inhibin/Activin βA-subunit was detected in the adrenal cortex, but the medulla and most of the pheochromocytomas were negative. It was also suggested that a clear βB-subunit expression in pheochromocytomas favors a benign nature [15]. Spencer et al. [16] reported immunoreactivities for βA- and βB-subunits in the adrenal cortex whereas the medulla was negative. However, Inhibin alpha is known as a positive immunohistochemical marker for adrenocortical tissue (benign and malignant adrenocortical tissue). Other markers are also known to be positive in adrenocortical tissue: bcl-2, calretinin, SF-1 [17, 18]. Some authors reported weak immunoreactivity for Inhibin alpha in 15–20% of pheochromocytomas [19, 20]. In our study, all the analyzed pheochromocytomas were negative for Inhibin alpha. The difference could be explained by the type of the used antibodies (we used monoclonal not polyclonal antibodies).
Primary known as a positive immunohistochemical marker for malignant melanoma, subsequent studies have revealed the utility of Melan A as a positive immunohistochemical marker for adrenocortical tissue (benign and malignant adrenocortical tissue) [21]. Durak et al. [1], Białas et al. [22] have studied the immunoreactivity of Melan A in pheochromocytomas and found no immunoreactivity of this marker in such tumors. In our study, we have also found no immunoreactivity of Melan A in either of the pheochromocytomas.

MNF116 is known as a positive immunohistochemical marker for benign and malignant epithelial tissue. We found no data regarding the expression of this antibody in pheochromocytomas. In our study, none of the pheochromocytomas showed immunoreactivity with MNF116. However, due to the small size of our study group, the statistical conclusions cannot be extended in general.

Conclusions

In our study, the analyzed pheochromocytomas did not stain with Inhibin alpha-subunit, Melan A or MNF116. Thus, we can say that inhibin alpha subunit, Melan A or MNF116 were not sensitive for the immunohistochemical diagnosis of pheochromocytomas but these markers can be of real help when we make the distinction between adrenal tumors because Inhibin alpha-subunit, Melan A or MNF116 were not sensitive for the immunohistochemical stain with Inhibin alpha-subunit, Melan A or MNF116.

Acknowledgments

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