S100 Expression in Oncocytoma and Chromophobe Renal Cell Carcinoma: An Immunohistochemical Study

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Abstract

Oncocytoma and chromophobe renal cell carcinoma may show a certain degree of histological overlap. The problem of differentiating both types is expected to increase because of the more frequent use of needle biopsies to diagnose small renal masses. In this study, the expression and the possible diagnostic utility of S 100 was evaluated in both oncocytoma and chromophobe renal cell carcinoma, using immunohistochemistry. Ten cases of oncocytoma and 10 cases of chromophobe renal cell carcinoma were used for the immunohistochemical study. Nine cases of chromophobe renal cell carcinomas (90%) showed no reaction with antibody to S 100. Only one chromophobe renal cell carcinoma (10%) was positive. Eight cases of renal oncocytomas (80%) gave positive reactions with antibody to S100, while two cases (20%) showed no reaction with antibody to S 100.

In conclusion: A significant difference in S 100 immunexpression between chromophobe renal cell carcinomas and oncocytomas has been detected (p<0.001) and it can be used as a differentiating point between both subtypes.

Key Words: S100 – Oncocytoma – Chromophobe renal cell carcinoma.

Introduction

ONCOCYTOMA and chromophobe renal cell carcinoma are two subtypes of renal cell neoplasms that may show a certain degree of histological similarity and the distinctive histopathological features cannot always be fully appreciated [1,2]. Oncocytoma is a benign tumor of uniform round/polygonal cells with abundant, intensely eosinophilic and granular cytoplasm with uniform small, round, central nuclei with evenly dispersed chromatin (Fig. 1). Oncocytoma constitutes 4-7% of adult renal epithelial tumors and its cell of origin is the intercalated cell of collecting duct. Chromophobe renal cell carcinoma is characterized by tumor cells with well defined cell borders, its cytoplasm is voluminous, pale and finely reticular, their nuclei are of low grade (Fig. 2). Chromophobe renal cell carcinoma constitutes 5% of adult renal epithelial tumors and its cell of origin is also the intercalated cell of collecting duct. The problem of differentiating both types is expected to increase because of the more frequent use of needle biopsies to diagnose small renal masses [3].

Immunohistochemical analysis is becoming more frequently useful to narrow the differential diagnosis or to arrive at a definitive diagnosis [4].

S-100 protein has been routinely applied for initial screening of various types of tumors, including, melanocytic tumors and neurogenic tumors. S-100 protein has been shown to have a broad distribution in human tissues, including renal tubules [5].

Potential utility of S-100 protein in renal cell neoplasms has not been extensively investigated. S 100 is calcium-binding protein of the EF-hand family, the mRNA for which has been found in renal cell neoplasms by PCR analysis [6].

In this study, the expression and the possible diagnostic utility of S 100 was evaluated in both oncocytoma and chromophobe renal cell carcinoma, using immunohistochemistry.

Material and Methods

Ten cases of oncocytoma and 10 cases of chromophobe renal cell carcinoma were used for the immunohistochemical study. The paraffin blocks...
of these tumors were retrieved from the Pathology Department, Faculty of Medicine, Cairo University and private laboratories within the period from January 2000 to December 2008.

The diagnoses were reviewed according to the WHO 2004 Classification of Tumors of the Urinary System and Male Genital Organs [7]. The clinical data were obtained from the pathology sheets of the patients.

Each paraffin block was re-cut by rotatory microtome at 5 microns thickness then mounted on glass slides to be stained by hematoxylin and eosin (H&E) for routine histopathological examination and on charged slides for immunostaining.

**Immunohistochemistry:**

After routine deparaffinization in xylene, the sections were hydrated through a series of graded alcohols (95%-70%), distilled water, and phosphate buffered saline (PBS) at pH 7.5. The slides were then immersed in 10 mM citrate buffer (ph 6) and were twice pre-treated by microwaving (oven 800W) for 4 then 8 minutes. Between each period of heating, evaporated fluid was replenished. After a 25 minute cooling period, the endogenous peroxidase activity was inhibited by incubation in 3% hydrogen peroxide (H₂O₂) for 5 minutes.

For immunohistochemistry, the tissue sections were incubated overnight at room temperature with a 1:50 diluted mouse monoclonal antihuman S100 antibody (clone: S1-61) (Santa Cruz Biotechnology, Inc. California, USA). The slides were then washed three times in Tris buffer. The Envision™ system, peroxidase, mouse (DAKO, Carpinteria, California, USA) was applied for 10 minutes, followed by washing in Tris buffer and using DAB (3,3 diamino-bezidine-tetrahydrochloride) as chromogen for 10 minutes. The slides were rinsed well in tap water for 5 minutes. The counter stain was performed using Mayer’s hematoxylin for 1-2 minutes and dehydrated in ascending grades of alcohol. Then slides were cleared in xylene for 3 changes, and then Canada Balsam and cover slips were applied. The negative control was carried out by omitting the primary antibody in each case.

**Evaluation of S100 immunostaining:**

S100 was evaluated as the positively stained neoplastic cells in a selected tumor section of each case of oncocytoma and chromophobe renal cell carcinoma. Cytoplasmic and/or nuclear staining was considered positive; S100 expression was considered negative in tumors completely lacking positive reactions [8].

**Statistical analysis:**

Data was collected, coded and analyzed using Windows Vista Home Premium, 2006 Microsoft corporation.

The Fisher exact test was used to compare S100 immunoexpression in renal oncocytomas vs chromophobe renal cell carcinomas. Results were considered statistically significant at a p value of 0.05 or less.

**Results**

Twenty cases comprised 13 males (65%) and 7 females (35%) were studied. Within the chromophobe renal cell carcinoma group; males constituted 7 cases and female cases were 3 only. On the other hand within the oncocytoma group; male cases were 6 and female cases were 4. Age of the patients ranged from 37 to 65 years.

**Immunohistochemical findings:**

Nine cases of chromophobe renal cell carcinomas (90%) showed no reaction with antibody to S100 (Fig. 3). Only one chromophobe renal cell carcinoma (10%) was positive.

Eight cases of renal oncocytomas (80%) gave positive reactions with antibody to S100, while two cases showed no reaction with antibody to S100 (Fig. 4). Most of them showed both cytoplasmic and nuclear immunostaining.

A significant difference in S100 immunoexpression between chromophobe renal cell carcinomas and oncocytomas was detected in this study (p<0.001).

Immunohistochemical S100 expression data are summarized in Table (1).

**Table (1): S100 immunoexpression.**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>No. of cases</th>
<th>S100 positive cases</th>
<th>S100 negative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromophobe RCC</td>
<td>10</td>
<td>1 (10%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td>10</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

p<0.001
Fig. (1): Oncocytoma H&E (x400).

Fig. (2): Chromophobe renal cell carcinoma H&E (x400).

Fig. (3): Chromophobe renal cell carcinoma showed no reaction with antibody to S100 (x400).

Fig. (4): Oncocytoma gave positive reaction with antibody to S100 (x400).

**Discussion**

Lines of treatment of renal cell neoplasms include radical nephrectomy, and minimally invasive approaches such as cryo- and radiofrequency ablation [9-12]. In line with the recently developed surgical sparing techniques, a preoperative diagnosis by needle-core biopsy is needed to guide management decisions. However, the specific morphological features for each type of tumors may not be appreciable in small samples and immunohistochemical analysis may be needed to support a definitive diagnosis [13,14].

S 100, has been found in renal cell neoplasms. This calcium binding protein is a member of the largest subgroup of the EF-hand proteins, which consists of several members that display amino-acid sequence homology ranging from 25 to 65% [15].

In this study, S 100 protein is expressed in 8 cases of renal oncocytomas (80%), and in only one case of chromophobe renal cell carcinoma (10%). I found significant statistical difference between S 100 immunoexpression in oncocytoma and chromophobe renal cell carcinomas ($p<0.001$).

The results of the present study go with study of Paolo, et al. [8] who worked on 51 cases of chromophobe renal cell carcinoma and 40 cases of oncocytoma. They found that only 6% of their studied chromophobe renal cell carcinomas were immunohistochemically positive for S 100, whereas 93% of the oncocytomas, were positive with most of them showing moderate to strong immunostaining.

The present results are also concomitant with Fan, et al. [6] who found positive immunoreactions with S 100 antibody in one (6%) out of 16 chromophobe renal cell carcinoma, and 13 (87%) out of 15 oncocytomas.

Barthelemy, et al. [16] stated that 14 out of 15 (93%) of oncocytomas were considered to be immunopositive. In contrast, all their nine studied chromophobe renal cell carcinoma were considered to be immunonegative. There was a significant difference in the positive percentages of staining.
of S 100 between these two subtypes ($p<0.01$). This result supports the results of the present study.

In contrast with all the previously mentioned studies, Cochand-Priollet, et al. [17] stated that positive reactions for S 100 protein, showed no significant difference between the two subtypes.

In conclusion, the potential usefulness of the monoclonal antihuman S 100 antibody in the differential diagnosis among chromophobe renal cell carcinoma versus renal oncocytoma was noticed.

References