NON-TUMOR CYSTIC LESIONS OF THE ADRENAL GLAND

Vesna Janevska¹, Vlado Janevski², Oliver Stankov³, Liljana Spasevska¹, Slavica Kostadinova-Kunovska¹, Julija Zhivadinovik⁴

¹ Institute of Pathology, Faculty of Medicine, Skopje, R. Macedonia
² University Clinic of Abdominal Surgery, Skopje, R. Macedonia
³ University Clinic of Urology, Skopje, R. Macedonia
⁴ Institute of Anatomy, Faculty of Medicine, Skopje, R. Macedonia

Corresponding Author: Julija Zhivadinovik, Institute of Anatomy, Faculty of Medicine, 50 Divizija 6, 1000 Skopje, R. Macedonia; Tel: +389 76 407 625; E-mail: zivadinovikj@yahoo.com

Abstract
Adrenal cystic lesions are uncommon but due to the improved radiologic imaging techniques their appearance seems to increase.

Material and Methods: We analyzed the clinical and radiological findings of 10 patients with adrenal cysts and the pathological features of the operative material. Standard dissection procedure and paraffin embedded tissue sections were made, stained by HE and immunohistochemically with CD34, CD 31, Factor 8, Podoplanin, CKWS and AE1/AE3

Results: The mean age of the patients was 40.6 years; female to male ratio was 2.3:1. All the cysts were diagnosed as cystic lesions radiologically except one. The most present clinical symptom was abdominal pain. The diameter of the cysts measured from 2 to 7 cm. Four of the cysts were diagnosed as pseudocysts and six as endothelial. Six cysts were lined by CD34⁺ and CD31⁺ cells, four were lined by Factor 8⁺ and podoplanin⁺ cells and four had no lining.

Conclusion: Endothelial cysts were more common cysts in our study and the immunohistochemical results suggested common vascular origin to all endothelial cysts and supported additional separation of angiomatous and lymphangiomathous adrenal vascular cysts.

Keywords: adrenal cysts, pseudocysts, CD 34, CD 31, podoplanin

Introduction
Adrenal cystic lesions (ACL) represent a rare entity and form a heterogeneous group of lesions with differences in the etiology and the clinical manifestations. The occurrence of ACL by autopsy is about 0.064–0.18% of general people [1–3]. Nowadays, due to the improved diagnostic methods like ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) their appearance seems to increase [4].

The adrenal cystic lesions have been classified traditionally into four categories by origin: pseudocysts, epithelial cysts, endothelial cysts and parasitic cysts [2, 5, 6]. Cystic tumors can be added to the group of adrenal cystic lesions. Adrenal tumors such as pheochromocytoma and cortical carcinoma may contain cystic changes due to hemorrhage or degeneration and they can mimic benign adrenal cysts. Such tumors must not be misdiagnosed as adrenal cysts [2].

Material and Methods
A retrospective analysis of the clinical, radiological and pathological data of 10 pati-
ents with adrenal cysts who underwent adrenalectomy in the last 6 years was made. We selected all adrenal cystic lesions received at our institution in a 6-year period. All of them turned out to be non-tumor, non-parasitic cystic lesions.

The clinical data, including radiological images and follow-up information were obtained from the patients clinical history files. For the pathological analysis archive material, paraffin blocks, and slides, and the pathological reports were used.

Adrenal glands were received at the Institute of Pathology in Skopje for histopathological examination. Standard dissection procedure of the operative material and paraffin embedded tissue sections were made. The sections were stained using the standard H&E and Van Gieson histochemical staining method. The immunohistochemical staining against CD34 (Dako, Code M7165), CD 31 (Dako, Code M0823), Factor 8 (Dako, Von Willerebrand Factor, Code M0616), Podoplanin (Dako, CodeIS072), SMA (Dako, Smooth Muscle Actin, Code M0851), CKWS (Dako, Anti-Cytokeratin Wide Spectrum Screening, Code Z0622) and AE1/AE3 (Dako, Cytokeratin, Code M3515) was performed using Avidin-Biotin Immunoperoxidase Complex technique. For the visualization of the antigen-antibody reaction, LSAB and En-Vision kits from DAKO were used.

Results
The age of the patients was from 28 to 58 years (median age 40.6 years). The female to male ratio was 2.3 : 1.

Eight of the patients presented had abdominal pain (one with severe pain due to cyst rupture) and 2 cysts were incidentally discovered.

All the patients underwent complete endocrinological evaluation which showed no significant deviations from the normal values, except one patient who was admitted to the hospital as an urgent case with acute abdomen due to the rupture of the cyst.

All adrenal cysts were diagnosed as cystic lesions of the adrenal gland by imaging techniques, except one which was diagnosed as a liver cyst (Figure 1, 2).

Figure 1a – CT of the right adrenal cystic lesion measuring 2 × 1 cm
Figure 1b – CT of the right adrenal cystic lesion measuring 2 × 1 cm
Figure 2 – The macroscopic appearance of the same cyst shown in Figure 1. The adrenal gland can be seen in the upper half of the photo. One half is hold by the pancetta and the other is placed next to the histo cassette. The cyst is cut in few pieces and the thin cystic wall can be seen in the lower part of the photo. The cystic wall is thin, smooth and glistening. A congested blood vessel can be seen in the cystic wall right in the photo.

The indication for surgery included abdominal pain in 7 patients; urgency for one patient and two patients who had incidentally identified adrenal cystic lesions had surgical indication from reason different then adrenal pathology.
The smallest cyst measured in diameter was 2 cm and the largest one measured was 7 cm. The clinical, radiological and some of the pathological findings are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Dimension</th>
<th>Clinical presentation</th>
<th>Radiological findings</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>f</td>
<td>4 cm</td>
<td>Abdominal pain, weakness</td>
<td>Adrenal cystic lesion</td>
<td>Pseudocyst</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>f</td>
<td>2 cm</td>
<td>Asymptomatic Incidentally identified</td>
<td>Adrenal cystic lesion</td>
<td>Vascular cyst</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>f</td>
<td>6 cm</td>
<td>Abdominal pain, nausea</td>
<td>Adrenal cystic lesion</td>
<td>Vascular cyst with calcifications</td>
</tr>
<tr>
<td>4</td>
<td>63</td>
<td>f</td>
<td>2 cm</td>
<td>Asymptomatic Incidentally identified</td>
<td>Adrenal cystic lesion</td>
<td>Vascular cyst with calcifications</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>f</td>
<td>5 cm</td>
<td>Acute abdomen</td>
<td>Adrenal vascular tumor</td>
<td>Pseudocyst ruptured</td>
</tr>
<tr>
<td>6</td>
<td>28</td>
<td>m</td>
<td>5 cm</td>
<td>Abdominal pain, discomfort</td>
<td>Adrenal cystic lesion</td>
<td>Vascular cyst with associated nodular hyperplasia</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>m</td>
<td>6 cm</td>
<td>Abdominal pain</td>
<td>Adrenal cystic lesion</td>
<td>Pseudocyst</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>f</td>
<td>7 cm</td>
<td>Abdominal pain, nausea, discomfort</td>
<td>Adrenal cystic lesion</td>
<td>Vascular cyst</td>
</tr>
<tr>
<td>9</td>
<td>58</td>
<td>m</td>
<td>2 cm</td>
<td>Abdominal pain</td>
<td>Adrenal cystic lesion</td>
<td>Pseudocyst with calcifications</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>f</td>
<td>3 cm</td>
<td>Abdominal pain</td>
<td>Liver cystic lesion</td>
<td>Vascular cyst</td>
</tr>
</tbody>
</table>

Six of the analyzed cysts were diagnosed as edothelial cyst and four cysts ware diagnosed as pseudocysts. Microscopically pseudocysts had thick connective tissue wall, mostly hyalinized with areas of calcifications. There were some adrenal cortical cells entrapped in the wall. We found smooth muscle bundles (positive for SMA) in the wall of 2 cases. Their lumina were filled with blood and the luminal surface was covered with clotted blood. All four pseudocysts were unilocular. No endothelial/epithelial cells were found to line the luminal surface. We found small blood vessels entrapped in the collagenous tissue of the cystic wall in all four cysts (Figure 3).

Figure 3 – Microphotography of a pseudo cyst.

a) A thick connective tissue wall mostly hyalinized with areas of calcifications near to the luminal surface (H.E. × 100);

b) There are adrenal cortical cells entrapped in the wall and many small blood vessels surrounding them (H.F. × 100)
The endothelial cysts had thin wall composed of collagenous tissue in which differentiated fibroblasts were seen. Five of the endothelial cysts were multilocular. The lumina of 4 cysts were filled with serous liquid or were empty; two cysts contained blood in its lumina. The lining cells were flattened, bland looking cells with oval elongated nuclei and could be recognized as endothelial cells in most of the cases. One multilocular endothelial adrenal cyst contained a great number of vascular spaces with different shaped lumina filled with blood. The morphological features were consistent with hemangioma (Figure 4).

All endothelial cysts contained small vessels in the capsule or in the fibrous septa which separated the cystic spaces in the multilocular endothelial cysts.

There were CD34 and CD31 positive (+) cells, lining the cysts in all of the cases. The lining cells of 4 endothelial cysts also showed positivity for Factor 8 immunostaining. Factor 8+ lining cells were found in 2 cysts filed with blood and 2 cysts filed with serous liquid (Figure 5).
The positive signal for Factor 8 of the lining cells was generally weak, compared to the positive control of the blood vessels endothelial cells present in the cystic wall. They also showed different signal intensity even in the same cases (Figure 6).

Four out of six endothelial cysts showed immunoreactivity with podoplanin in the lining cells. All of them contained proteinaceous material in their lumina. Small lymphatic vessels lined with podoplanin+ cells were present in the cystic wall or in the cystic septa, as well as small blood vessels lined with podoplanin- endothelial cells. We could not find any specific order in the arrangement of the small vessels lined with podoplanin+ and podoplanin− cells, although their arrangement in some areas where they were closely packed might suggest vascular hamartomatous lesion (Figure 7).

The pathologic and histopathologic findings are presented in Table 2.
Table 2

Pathological findings

<table>
<thead>
<tr>
<th>Cyst</th>
<th>No of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Epithelial</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Pseudocyst</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>Thickness of the wall</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 0,1</td>
<td>5</td>
<td>50%</td>
</tr>
<tr>
<td>≤ 0,2</td>
<td>2</td>
<td>20%</td>
</tr>
<tr>
<td>≤ 0,3</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>Muscle bands in the wall</td>
<td>2</td>
<td>20%</td>
</tr>
<tr>
<td>Calcifications</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>Cortical cell nests in the wall</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Content of the cyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Liquid</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>Cavities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilocular</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>Multilocular</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Other pathology</td>
<td>1</td>
<td>10%</td>
</tr>
<tr>
<td>CD34 positivity</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>CD31 positivity</td>
<td>6</td>
<td>60%</td>
</tr>
<tr>
<td>Factor 8</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>Podoplanin</td>
<td>4</td>
<td>40%</td>
</tr>
<tr>
<td>CKWS positivity</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>AE1/AE3 positivity</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>No lining cells</td>
<td>4</td>
<td>40%</td>
</tr>
</tbody>
</table>

All ten patients are still alive. They did not have postoperative complications or any adrenal symptoms.

Discussion

Patients with adrenal cysts are in their fifth to sixth decade, more frequent of female gender [1]. In our study the average age of the patients was 40.6 years, and, the female to male ratio was 2.3 : 1. Majority of the patients have unilateral lesion although the bilateral lesions have been described in about 8–15% of cases. In our series all analyzed cysts were unilateral.

The adrenal cystic lesions are usually presented with abdominal pain or can be asymptomatic or they may manifest with nonspecific symptoms, like headache, palpitations, fever, hypertension and ureteral calculus. Usually, the abdominal or flank pain, gastrointestinal symptoms and the palpable mass are the main clinical manifestations [1]. In eight of our patients the main symptom was abdominal pain.

Non-tumor adrenal cysts are usually well circumscribed and encapsulated with size range from 1.4 to 33 cm in diameter. The mean size of the symptomatic cysts is usually larger than that of the asymptomatic cysts [1, 3]. The large cysts have a tendency to develop such complications as intracystic hemorrhage and rupture that can manifest as a surgical emergency [3, 7–10]. Just one case with cyst rupture was observed in our study.

The radiographic findings are not specific, but the cystic nature and the liquid content can be detected by ultrasonography, computed tomography and magnetic resonance imaging. US reveals well marginated, anechoic lesions and can easily identify uncomplicated cysts, however, it had limitation in distinguishing the source of the retroperitoneal cysts and the adrenal mass accompanied with cysts [3–5]. CT scans demonstrate hypodense, non-enhancing masses. US and CT scans can reveal peripheral and curvilinear calcifications of the cystic wall in 15% of cases. Small adrenal cysts with a wall diameter of less than 3 mm and water attenuation in the CT scan are typically considered to be benign lesions. Uncomplicated adrenal cysts present as non-enhancing masses with smooth thin walls [4]. On MRI, simple cysts of AL are visible as low intensity signals on T1-weighted images and high intensity signals on T2-weighted images. In contrast, complex cysts, such as those characterized by hemorrhage or, infection, appear as high signal intensity signals on both T1- and T2-weighted images. Our cases were diagnosed by US and CT. However, the imaging findings are frequently ambiguous because of the adjacent organs [11, 12].

Cystic lesions in the adrenal gland are uncommon and since the adrenal non-tumor cysts are also very rare, the reference data in the literature are scarce and the series are small with the exception of Sebastiano et al. who reported 12 pseudocysts, 2 endothelium-derived cysts and 17 epithelium-derived cysts in a period of 20 years [13].

Most authors, as we have already mentioned above, have analyzed small series of adrenal cyst and have classified them as pseudocysts, endothelial cysts and epithelial cysts (excluding parasitic ones).

According to Xiao Lyu et al. ACL can be classified as endothelial cyst, 45%; pseudocyst, 39%; epithelial cyst, 9%; parasitic cyst, 7% [2, 3, 14].

Immunohistochemical and electron microscopy investigations suggest vascular origin of
the adrenal pseudocysts and the endothelial cysts [1]. According to the literature, these two types of cysts are considered as variants of the vascular adrenal cysts and account for the majority of the cases of adrenal non-neoplastic cysts [1–3, 11, 12]. Various theories have been suggested concerning the pathogenesis of the adrenal vascular cysts, including the origin from a preexisting vascular hamartoma, ectasia of lymphatic channels, or origin secondary to the intraparenchymal hemorrhage [1].

The endothelial cysts can be further subdivided into angiomatous, lymphangiomatous and hamartomatous cysts. Most endothelial cysts are lymphangiomatous, and they are usually composed of multilocular, thin-walled cystic lesions and contain yellow-tinged serous fluid [11, 14]. Typically, they are formed by irregular dilated spaces within endothelial lined cavities. The endothelial cells lining these cavities are immunoreactive for the vascular cell markers such as podoplanin (D2-40), CD31, CD34, and factor VIII–related antigen. The lining of some of the endothelial cysts reported in the literature reacted only weakly for factor VIII–related antigen, but it stained strongly for collagen type IV [11, 15]. This staining pattern, along with the lack of hemorrhage in endothelial cysts, suggests lymphatic differentiation.

In our study, six of the analyzed cysts were diagnosed as endothelial cysts and significant CD31, CD34 immunoreactivity of the lining cells was observed in all of them. The lining cells of 4 endothelial cysts also showed positivity for Factor VIII immunostaining, but it was generally weak. Two of these Factor VIII positive cysts were filled with blood and two of them contained serous liquid in their lumina. Four endothelial cysts showed immune expression of podoplanin in the lining cells. None of these four cyst contained red blood cells in their lumina; they contained proteinaceous material.

The panel of the antibodies applied in differentiating endothelial adrenal cysts is generally limited to the above mentioned markers, but there are reports which suggest that LYVE-1 (Lymphatic Vessel Endothelial Hyaluronan receptor 1) and Prox-1 antibodies, specific for the lymphatic endothelial cells, would be also appropriate markers for detecting the lymphangiomatous endothelial adrenal cysts [16, 17].

Microscopically, pseudocysts have a wall of dense, hyalinized connective tissue that may have focal calcifications or metaplastic bone formation. Entrapped cortical cells are identified in the cyst wall. In some cases, smooth muscle is detected within the wall of the cyst, and it seems to be continuous with the smooth muscle of the adrenal vein. Similarly, in few cases there are irregular endothelial-lined spaces within the residual cortex and the cyst wall itself. No endothelial lining is detected in the cyst wall. The contents of the cyst are clotted blood and fibrinous material that may contain islands of viable or necrotic cortical cells. Staining results similar to those of factor VIII antigen have been obtained in the residual foci of the flattened cells lining the inner wall of some pseudocysts, lending support to the notion that the pseudocysts are of vascular origin [1]. The immunohistochemical staining for epithelial markers (EMA, AE1/AE3, CAM 5.2) is negative in both types of adrenal vascular cysts [1]. Four cysts in our series were diagnosed as pseudocysts. Microscopically they had thick connective tissue wall, mostly hyalinized with areas of calcifications. We found smooth muscle bundles in the wall of 2 cases. Their lumina were filled with blood and the luminal surface was covered with clotted blood. No endothelial/epithelial cells were found to line the luminal surface. We found small blood vessels entrapped in the collagenous tissue of the cystic wall in all four cysts.

The epithelial cysts are lined by a single layer of cytokeratin positive cells and have been subdivided into three groups based upon the proposed theories of pathogenesis, that is still unclear and can be subdivided into glandular or retention cysts, cystic adenomas and embryonal cysts [3, 14, 18]. We haven’t diagnosed any epithelial cyst in our series.

In the presence of symptoms, endocrine abnormalities, complications, suspicion of malignancy and large size, surgical excision is indicated. Patients after surgical resection should be followed up closely especially in functional cysts and when the histopathology showed a cystic tumor [3]. In our study, surgical intervention was the main management method for adrenal cyst.
Conclusion
Our results of small series suggest common vascular origin of the endothelial cysts which can be further subdivided into hemangiomaticous and lymphangiomaticous cysts. All of them, including the pseudocysts show nonspecific radiological findings and clinical manifestations and are curable with surgical intervention.

REFERENCES

Резиме
НЕТУМОРСКИ ЦИСТИЧНИ ЛЕЗИИ НА НАДБУБРЕЖНАТА ЖЛЕЗДА
Весна Јаневска1, Владо Јаневски2, Одимер Станков3, Лијана Спасевска4, Славица Костадинова-Куновска5, Јулија Живадиновик6

Цистичните лезии на надбубрежната жлезда се ретки промени, но развојот на современите радиолошки и имунолошки техники овозможува нивно лесно дијагностицирање, па изгледа како постојано ќе се јавуваат.

Материјал и методи: Анализирани се клиничките и радиолошкиот наод од 10 пациенти со цистични промени на надбубрежната жлезда, како и резултатите од патохистолошката анализа на оперативниот материјал, направен со стандартна процедура на дисекција и просечен на тканино вкалапено во парафин. Применети се методите за имуностохемиски боења со CD34, CD 31, Фактор 8 и CD 20, апоптоза и имунохистохемиски боења со CD34, CD 31, Фактор 8, Јулија Живадиновик

Резултати: Средната возраст на пациентите изнесуваше 46,0 години; односот височина ширина беше 2,3:1. Сите цисти, со иску-
Non-tumor cystic lesions of the adrenal gland

...чок на една, радиолошки беа дијагностицирани како цистични лезии. Како најчест клинички симптом беше присутна абдоминална болка.

Дијаметарот на цистите изнесуваше од 2 до 7 cm. Хистолошки, четири од цистите беа дијагностицирани како псеовдоксисти, а шест како ендотелни цисти. Шест цисти беа обложени со CD34+ и CD31+ клетки, четири со Фактор 8+ и подопланин+ клетки, а четири цисти не беа воопшто обложени.

Заклучок: Во нашата студија ендотелните цисти беа позастапени. Резултатите од имунохистохемиската анализа укажуваат на заедничко васкуларно потекло на сите ендотелни цисти и го поддржуваат дополнителното раздвојување на ангиоматозни и лимфангiomатозни цисти на надбубрежната жлезда.

Ключни зборови: надбубрежна жлезда, псеовдоксисти, CD 34, CD 31, подопланин