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CONCURRENT PERITONEAL MESOTHELIOMA AND UTERINE ADENOCARCINOMA IN A RABBIT (ORYCTOLAGUS CUNICULUS)

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Summary: In this case report, peritoneal mesothelioma, and uterine adenocarcinoma were described with clinical, morphological, and immunohistochemical findings in a five year-old female rabbit. Clinical abnormalities included anemia, anorexia, dull demeanour, and droopy. Abdomen was distended with a few fluid, and anechoic areas were seen between all hepatic lobes on ultrasonography. At necropsy, nodules were seen as multiple firm, round, white-gray 0.5 cm to 1.5 cm in diameter in the peritoneal surfaces of the omentum, intestine, uterus, diaphragma. Also, the right uterine mucosa was thickened, and it was included multiple round, hard, grayish nodules 2 mm to 7 mm in diameter and their metastatic nodules were seen in the lungs. Microscopically, there were seen two types tumors: The first one, mesothelioma in the periton and peritoneal surfaces was composed of round to polyhedral large epithelial cells with round to oval nuclei, and eosinophilic vacuolated cytoplasma. They were formed solid nests or projecting in a papillary pattern. The neoplastic cells were stained positively with Alcian blue, and also, antibodies to kalretynyn, vimentin and cytokeratine. The other one, uterine adenocarcinoma and metastatic adenocarcinomas to lung were consisted of large and pleomorphic cells and had abundant cytoplasm. The neoplastic cells in the endometrium and lungs were reacted with antibodies to carcinoembriogenic antigen and S-100 protein.

Key words: Mesothelioma, adenocarcinoma, periton, uterus, rabbit.

Bir Tavşanda (*Oryctolagus cuniculus*) Rastlanan Peritoneal Mezotelyoma ve Uterus Adenokarsinomu

Özet: Bu olguda 5 yaşlı, dişi bir tavşanda gözlenen peritoneal mezotelyoma ve uterus adenokarsinomu bulguları klinik, morfolojik ve immunohistokimyasal olarak tanımlandı. Klinik olarak hayvan anemik, anorektik ve halsizdi, karın şişkindi. Ultrasonografide tüm hepatik loblar arasında anekoik alanlar görüldü. Nekropside omentum, barsak ve uterus serozası ile diyaframada 0.5-1.5 cm çapında, çok sayıda, sert, yuvarlak gri-beyaz nodüller vardı. Ayrıca, kalınlaşmış olan sağ uterus mukozası üzerinde de 2-7 mm çapında, çok sayıda, sert, yuvarlak, grimsi nodüller gözlendi. Akciğerlerde de yaklaşık 1 mm çapında benzer nodüllere rastlandı. Mikroskopik incelemede ise iki farklı tümörle karşılaşıldı. Bunlardan biri periton ve serosal yüzeylerde saptanan mezotelyoma; diğeri ise uterusta adenokarsinom ve bunun akciğer metastazı idi. Mezotelyoma, solid ya da papiller dizilimli; oval ya da yuvarlak çekirdekli, eozinofilik vakuoler sitoplazmalı yuvarlak veya polihedral görünümlü hücrelerden ibaretti. Bu hücreler Alcian blue, anti-kalretynyn, -vimentin ve -cytokeratine ile pozitif reaksiyon verdi. Uterus adenokarsinomu ve akciğer metastazı ise büyük, geniş sitoplazmalı pleomorfik hücrelerden oluşmuştu. Bu hücreler ise anti-carcinoembriogenic antigen ve S-100 proteini ile pozitif reaksiyon gösterdi.

Anahtar Sözcükler: Mezotelyoma, adenokarsinom, periton, uterus, tavşan.

INTRODUCTION

Mesotheliomas are rare occuring tumors arise from the mesothelial lining cells of coelemic cavities (pericardial, pleural, and peritoneal) or the tunica vaginalis. They have been reported in man and in a variety of animal species; greatest frequency in cattle, sheep, horses and dogs[1-4]. There is only one case reported in rabbits[5]. Mesotheliomas occur in newborn or very young cattle and sheep whereas in other species adult or aged animals have been reported[4]. They can be solitary or multiple and arises from the widespread habit of classifying mesotheliomas into epithelial, mesenchymal, and biphasic categories. Epithelial mesotheliomas are defined as resembling adenocarcinomas, mesenchymal mesotheliomas as resembling sarcomas, and biphasic mesotheliomas as resembling both, as in synoviomas[4,6]. Epithelial mesotheliomas are frequently difficult to distinguish from adenocarcinoma and that fibrous mesotheliomas are difficult to distinguish from other sarcomas[6-9]. It is evident from the number of studies seeking diagnostically useful criteria from ultrastructural or immunoperoxidase technics[5-7,9,10]. Metastasis is rarely reported and generally to the regional lymph nodes, bone marrow and viscera[4,11]. Adenocarcinoma of the uterus which was founded in the report, is a malignant epithelial neoplasm which arises from the endometrium. It is primarily a human neoplasm but most frequently seen in the cow, cat, rabbit, and dog[4,12]. There is a conspicuous rarity in other species[1]. It is generally due to over stimulation of estrogens[13] and metastasis occurs in the lungs, brain, ovary, liver, kidney, lymph nodes, and eye[14].

The case report describes clinical, morphological, and immunohistochemical features of a case of peritoneal mesothelioma and uterine adenocarcinoma in a rabbit (Oryctolagus cuniculus).

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CASE REPORT

A five year-old female, white rabbit (Oryctolagus cuniculus) was presented with 3 day history of anorexia, dull demeanour, and droopy. Supportive therapy was not beneficial. Symptomatic therapy included oral spironolactone-hydrochlorothiazide (2-4 mg/kg/12h), intramuscular ampicilline (10-20 mg/kg/12h), furosemide (2 mg/kg/day), and B-complex vitamins (0.5-1 ml/day).

Clinically, body temperature was normal, and mucous membranes were anaemic. The abdomen was very distended and palpable fluid pattern was detected in cauda ventral abdomen. This fluid was identified by Rivolta test as exudate. On the abdominal ultrasonografic evaluation, anechoic areas were seen between all hepatic lobes and it was diagnosed ascites. The serum values were urea 51.2 mg/dl, and creatinin 2.27 mg/dl. The initial clinical impression was that of advanced ascites due to liver insufficiency according to the clinical findings. The animal died during examination despite continued supportive treatment and necropsied according to owner's request.

At necropsy, significant abnormalities were confined to the thoracic and especially abdominal cavities. The abdominal cavity contained a 600 cc reddish fluid. The peritoneal surface of the omentum, intestine, uterus, and diaphragma were covered by a layer of multiple firm, round, white-gray nodules from 0.5 cm to 1.5 cm in diameter. On section, there were no lobulations. On the otherhand, the right uterine wall was thickened. There were multiple, hard, grayish nodules from 2 mm to 7 mm in the area. At the same time, nodules were seen both superficially and deep in the lungs. They were well defined, round, grayish-yellow nodules from 1 mm to 3 mm in diameter in lobes.

The masses existing in the abdominal cavity and lungs were fixed in 10 per cent neutral buffered formalin, routinely processed, embedded in paraffin, and stained with hematoxyline and eosin, Periodic acid Schiff, and Alcian blue stains. Additionally, selected sections of the masses and lungs were stained by the standard avidin-biotin complex peroxidase (ABC-P) method (Cadenza Tags / Shandon), with minor modifications. After dewaxing and rehydration, endogenous peroxidase activity in tissue sections was blocked by applying hydrogen peroxide 0.3 per cent methanol for 20 min. followed by treatment pronase for 10 min. at 40 C. An incubation with normal goat serum was taken place for 20 min. at 40 °C. Then the sections were incubated for 1 hour at 40 °C with primary antibodies (anti-vimentin, -actine, -cytokeratine, -S-100 protein, -kalretynyn, and -carcinoembriogenic antigen (CEA)) by obtained from Dako and used at a dilution 1:100 and 1:500. Next, the sections were incubated under the same conditions, biotinylated goat anti-rabbit IgG and streptavidin-peroxidase reagent for 20 min. at 40 °C. Colour was developed by a final incubation with 3-amino-9-ethylcarbazole for 5 min. at room temperature. Following every incubation step, sections were throughly washed with phosphate buffered solution, except the step with normal goat serum. The sections were counter stained with haematoxylin. Negative controls were run by omitting the primary antibodies.

Microscopically, it was observed that the nodules consisted of two types neoplastic cells. One of them was composed of masses of round to polyhedral large epithelial cells with round to oval nuclei (Figure 1,2), and eosinophilic cytoplasma which some of these cells vacuolated. In some areas, the nuclei was spindle shaped with distinct nuclear membrane. They were formed solid nests or projecting in a papillary pattern from the peritoneal surface of the omentum, intestine, uterus, and diaphragma. Neoplastic cells were also penetrated to the tunica muscularis of the uterus, intestine, and diaphragmatic muscle in some areas. Irregular areas of necrosis was noticed. There was moderate connective tissue in the around tumor cell sheets. In many areas there was a sharp transition from normal to tumor. The neoplastic cells were stained positively with Alcian blue, and also, antibodies to kalretynyn, vimentin and cytokeratin by immunoperoxidase method (Figure 3). As a result, the tumor was considered to be epithelial mesothelioma.

The other type of neoplastic cells were seen in the endometrium (Figure 4a) and lungs (Figure 5). These cells were large and pleomorphic and had abundant cytoplasma. They contained well differentiated gland and distinct lumen formation which was filled with secret. In some areas, mitotic figures, hemorrhagia, and calcification were rarely seen. The stroma showed fibrosis and infiltration on inflammatory cells. The neoplastic cells in the endometrium (Figure 4b) and lungs (Figure 6) were reacted with antibodies to CEA and S-100 protein by immunoperoxidase method. According to these, it was called adenocarcinoma, and the metastasis to the lungs was also observed morphologically similar to the uterine adenocarcinoma.

DISCUSSION

The etiology of mesotheliomas is poorly understood although the clinical and pathological features of the tumor have been examined[15]. There is considerable epidemiological evidence linking asbestos inhalation with human pleural mesotheliomas. They develop in experimental animals following inhalation, direct

intrapleural or intraperitoneal implantation of several types of mineral fibers, including different forms of asbestos, zeolite, fiberglass, SV40, MC29 avian leucosis virus, and polyoma virus[2,3,16-19]. In spontaneous mesotheliomas of animals no apparent epidemiologic pattern exists, except in calves, in which the tumors are mostly congenital[4,5]. The same as the studies, the tumors found spontaneously in this case, their aetiology are not clear.

The major morphological features were similar to those previously described for most mesothelioma[1,2,5,14,20] and adenocarcinoma[12,14] cases while in this case. Mesotheliomas may at times be difficult to distinction grossly and microscopically from metastatic or primer carcinoma especially pulmonary carcinoma[8,9]. A variety of histochemical staining techniques have been traditionally used in an attempt to establish the diagnosis[21,22]. According to the studies, the demonstration of hyaluronic acid was an indication of a mesothelioma whereas the presence of neutral mucins was a diagnostic feature of adenocarcinoma by histochemical stains. Furthermore, Chiu et al.[23] indicates that the histochemical finding of hyaluronic acid cannot be used as an absolute criteria for the diagnosis of mesothelioma. Electron microscopy can also be helpful, but the demonstration is long and difficult[24]. So, a variety of immunohistochemical staining techniques have been traditionally used in an attempt to establish the diagnosis in recent years. Most investigators agree that immunohistochemistry of diverse antigenic substances has been claimed to be of value in the differential diagnosis of carcinoma from mesothelioma[7-10]. CEA of them is the most widely accepted of the immunohistochemical markers used in the differential diagnosis between mesothelioma and adenocarcinoma, in general, mesotheliomas are negative or only weakly reactive for CEA whereas many carcinomas stain strongly[7,9]. In the study, while staining positively with Alcian blue, anti-kalretynyn, -cytokeratin and -vimentin as it stained negatively with anti-CEA and -S-100 protein, the diagnosis of mesothelioma was put to the tumor. Adenocarcinoma in uterus and its metastatic nodules in lungs stained positively with anti-CEA and -S-100 protein.

As a result, encountering two tumors that are seen rarely and frequently in a rabbit, and also coming across a tumor combination which is seen rarely[25-27] makes the case interesting.

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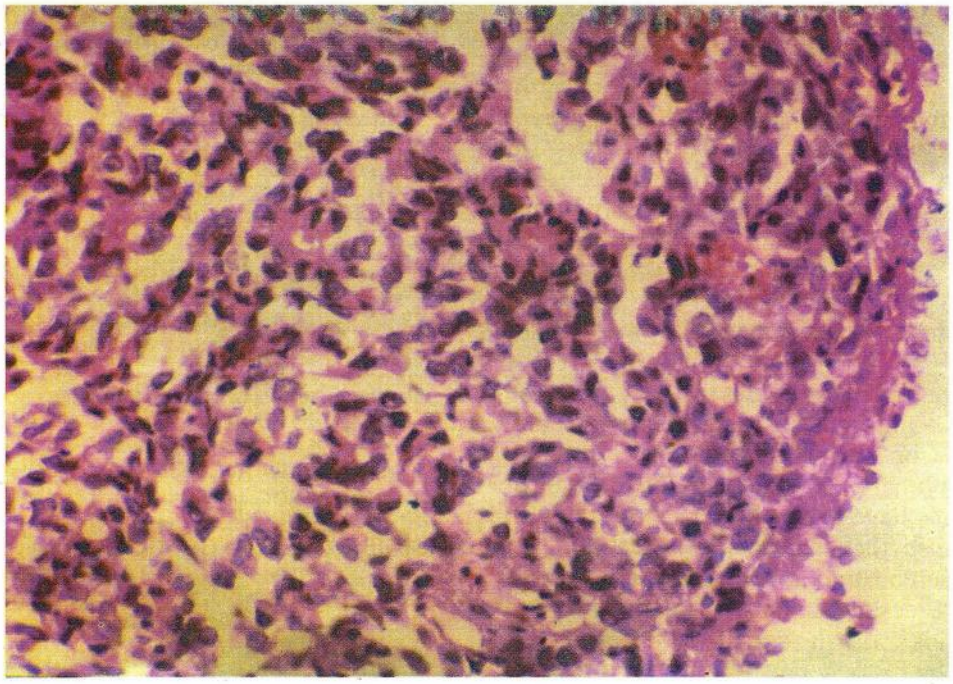

Figure 1. Solid nests of neoplastic mesothelial cells (HE X 200).
Resim1. Neoplastik mezotelyal hücrelerde solit nestler.

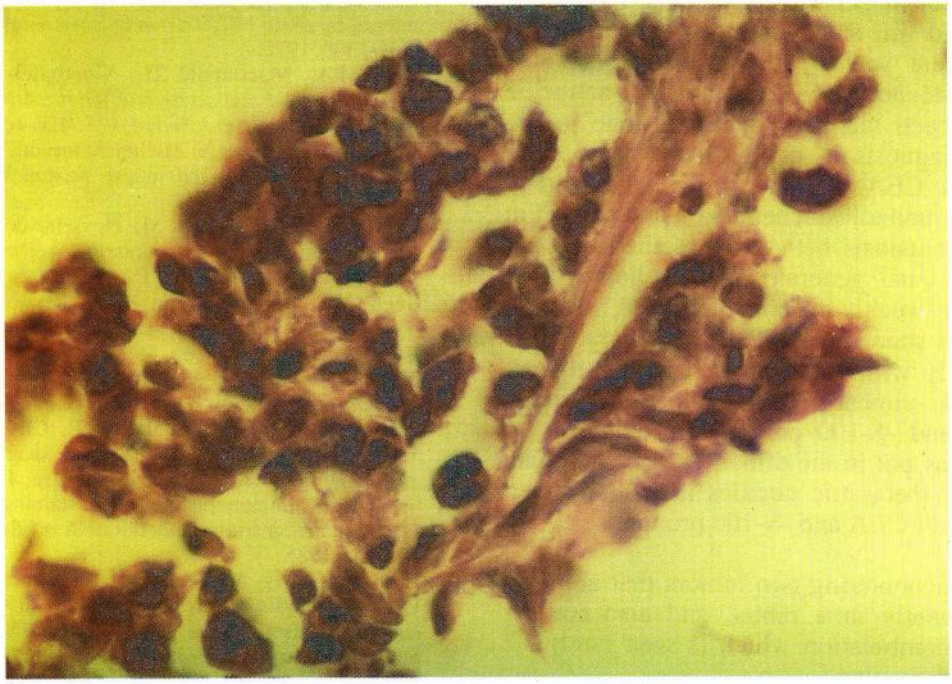

Figure 2. Cytological detail of neoplastic mesothelial cells (HE X 400).
Resim2. Neoplastik mezotelyal hücrelerde sitolojik detay.

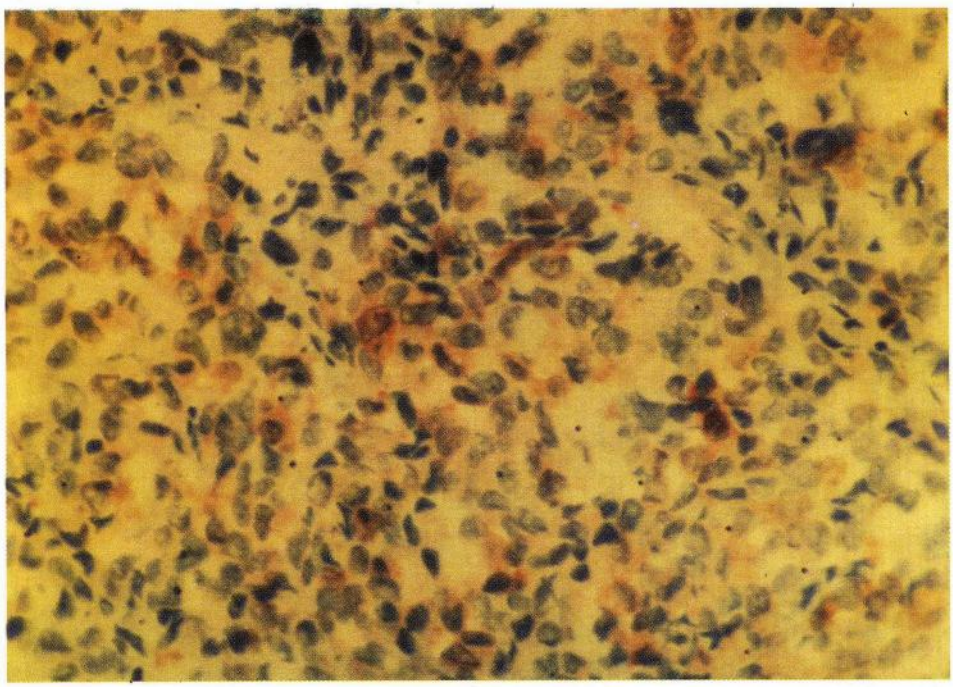

Figure 3. Staining with anti-kalretynyn in many neoplastic cells of mesothelioma (ABC-P stain X 200).
Resim3. Mezotelyomdaki neoplastik hücrelerin çoğunda anti-kalretinin ile boyanma.

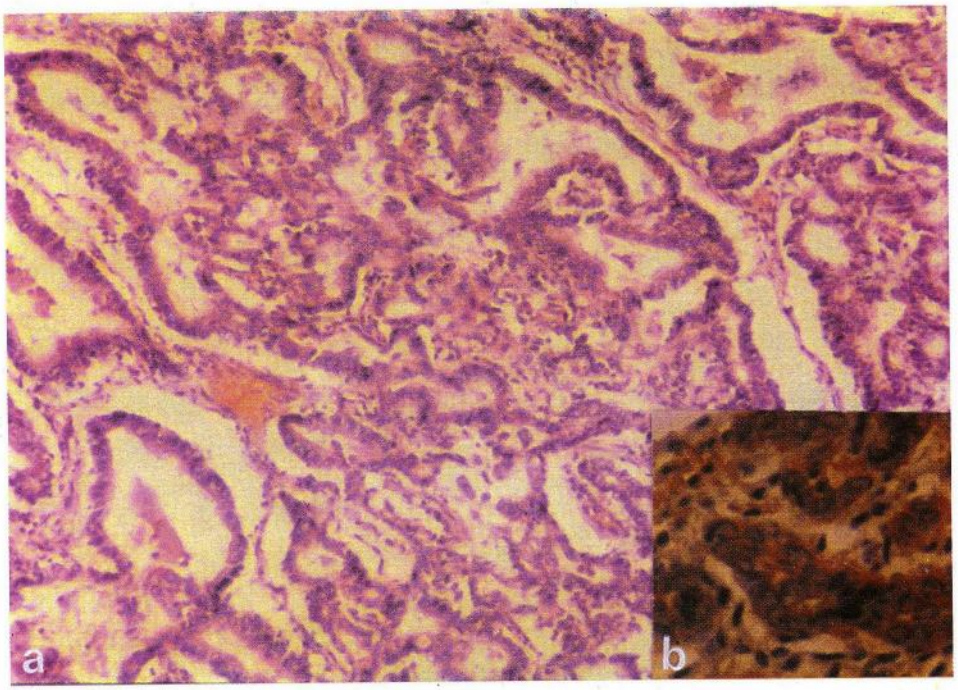

Figure 4. **a-** Pleomorphic neoplastic cells from the glands in uterus (HE X 100).
b- Staining with ante-CEA in many tumor cells of uterine adenocarcinoma (ABC-P stain X 200).
Resim4. a- Uterus adenokarsinomasındaki tömür hücrelerinin çoğunda anti-CEA ile boyanma.

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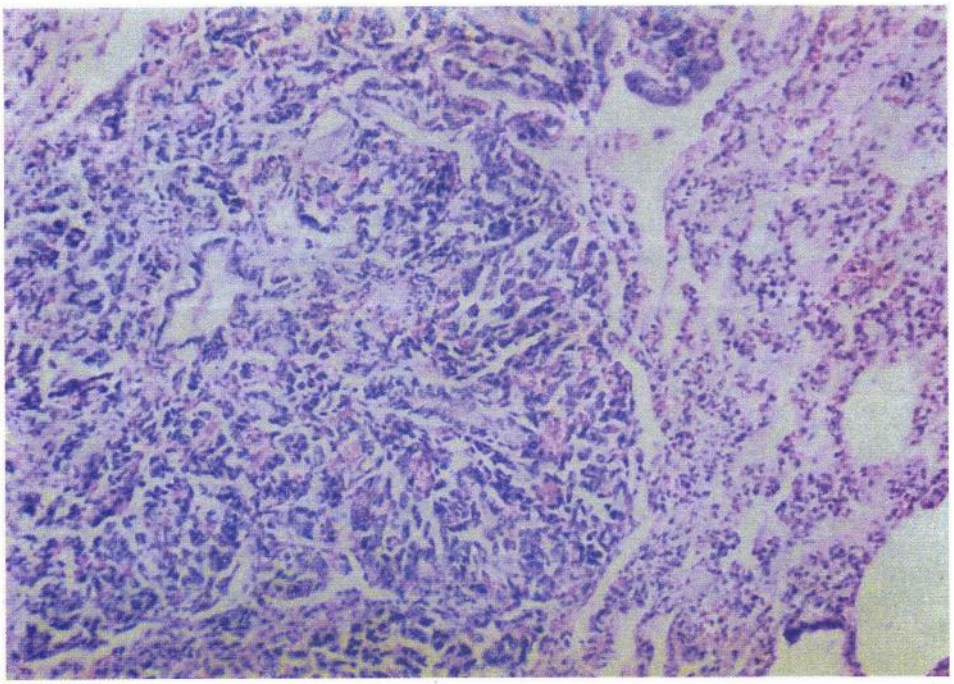

Figure 5. Metastatic nodule of adenocarcinoma in lungs (HE X 80).
Resim5. Akciğerlerde metastatik adenokarsinom nodülleri.

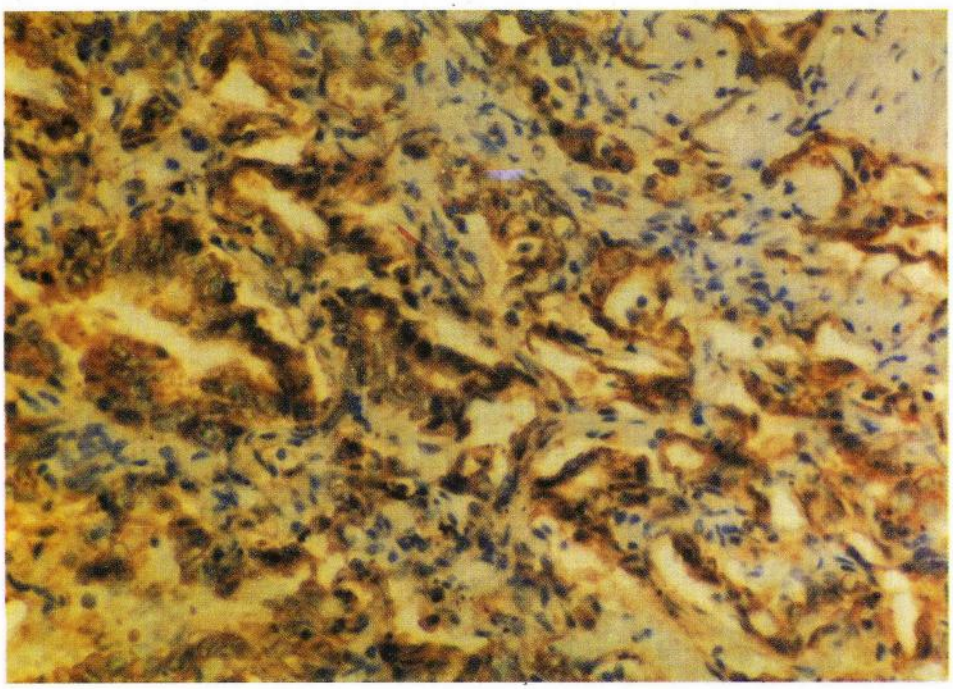

Figure 6. Staining with anti-CEA in many tumor cells of metastic nodule in lungs (ABC-P stain. X 200).
Resim6. Akciğerlerdeki metastatik nodüllerdeki tümör hücrelerinde anti-CEA ile reaksiyon.