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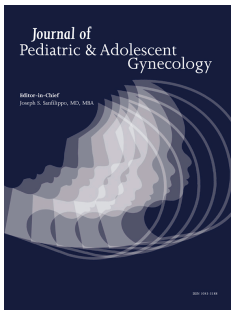

Small cell carcinoma of ovary, hypercalcemic type: cytologic, histopathologic and immunohistochemical landscapes of a rare case

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Title: Small cell carcinoma of ovary, hypercalcemic type: cytologic, histopathologic and immunohistochemical landscapes of a rare case

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Abstract

Background: Small cell carcinoma of ovary, hypercalcemia type (SCCOHT), also known as the malignant rhabdoid tumor of the ovary, is a rare and highly aggressive malignancy affecting younger women. The pathogenesis involves mutations in *SMARCA4/BRG1 and/or SMARCA2/BRM*.

Case: A 10-year-old girl presented with lower abdominal pain and a mass for the past 2 weeks. She underwent ultrasound-guided fine needle aspiration and core needle biopsy from the pelvic mass followed by surgery. Based on the characteristic morphologic and immunohistochemical features, a diagnosis of SCCOHT was rendered. She was started on chemotherapy, however, succumbed to the disease.

Summary and Conclusion: SCCOHT is a rare but highly aggressive ovarian malignancy with poor clinical outcome. A high index of clinical suspicion and adequate knowledge of its characteristic cytologic, histopathologic and immunohistochemical features are essential for accurate diagnosis of SCCOHT.

Title: Small cell carcinoma of ovary, hypercalcemic type: cytologic, histopathologic and immunohistochemical landscapes of a rare case

Introduction

Small cell carcinoma of ovary, hypercalcemic type (SCCOHT) is a rare and aggressive malignancy affecting women in a wide age range.[1] The pathogenesis involves mutation in *SMARCA4/BRG1 and/or SMARCA2/BRM*. The disease follows a rapid downhill course with majority of the patients showing disease progression despite aggressive treatment. Owing to the non-specific clinical presentations and overlapping radiologic features, which are often similar to other ovarian malignancies, establishing a clinical diagnosis of SCCOHT is extremely challenging. However, two-third of these cases may have associated hypercalcemia.[2]

Definitive diagnosis requires microscopic examination of the tumor tissue. Histopathologically, the tumor exhibits a highly undifferentiated morphology, mimicking many other ovarian tumors, which may further compound the diagnostic difficulty. Immunohistochemistry can help in reaching to an accurate diagnosis in such cases. Lack of awareness of its characteristic morphologic and immunohistochemical features can often lead to the tumor being misdiagnosed as other ovarian neoplasms including, include granulosa cell tumor, small cell carcinoma ovary, pulmonary type (SCCOPT), dysgerminoma, non-Hodgkin's lymphoma and undifferentiated carcinoma. This often carries significant clinical implications as the prognosis and therapeutics vary. Additionally, the cytological findings of this uncommon entity have only been sparsely described in the published literature. Herein, we present a case of SCCOHT in an adolescent and describe its cytological, histopathological and immunohistochemical features.

Case report

A 10-year-old girl presented with pain lower abdomen and fever for 15 days and swelling in the lower abdomen for 7 days. The pain was continuous, dull-aching and fever was intermittent, low grade. There was no history of respiratory distress, vomiting, bone pain, bladder or bowel symptoms or other systemic symptoms. Her body weight and height were below 3 standard deviations for her age with body mass index of 13.57. Clinical examination revealed a large abdominal mass felt in the left iliac fossa, extending to the umbilical and left hypogastric regions. Laboratory investigations revealed a haemoglobin of 10.4 g/dl, with normal leucocyte and platelet counts. Liver and renal function tests were within normal limits. Tumor markers including alpha fetoprotein and beta-hCG (human chorionic gonadotropin) were normal. Contrast-enhanced computerized tomography (CECT) of the abdomen showed a large, heterogeneously enhancing space occupying lesion in the pelvis measuring 10.5x8.4x6.7 cm, with extensive necrosis and no calcifications. The lesion was indenting bladder and anterior abdominal wall with preserved interface. Uterus and left ovary could not be visualised separately. CECT thorax and bone scan were normal.

An ultrasound-guided fine needle aspiration cytology (FNAC) was done from the ovarian mass. The smears prepared were cellular and showed tumor cells in clusters as well as scattered singly (Figure 1A). Majority of the tumor cells exhibited moderate degree of nuclear pleomorphism, with coarsely clumped chromatin, small nucleoli and indistinct cytoplasmic borders (Figure 1B, C). Some of the cells were larger, with moderate to abundant amount of dense eosinophilic cytoplasm with eccentric nuclei having prominent nucleoli, imparting a rhabdoid appearance (Figure 1F). Section from the cell-block also recapitulated similar cytomorphology (Figure 1D-F). Areas of necrosis were also noted in the cell-block section (Figure 1D). Based on these features, differential diagnoses of rhabdomyosarcoma and malignant rhabdoid tumor were offered.

Immunocytochemistry (ICC) was performed on the cell-block, and the tumor cells showed positivity for vimentin, CD99 (paranuclear dot positivity) and were negative for myogenin, myoD1 and desmin (Figure 1G-I). Based on these findings, the possibility of rhabdomyosarcoma was excluded. True-cut biopsy suggested the possibility of abdominal primitive neuroectodermal tumour (PNET). Hence, she was started on intergroup INT-0091 protocol for Ewing family of tumours comprising of alternating 2 weekly cycles of VDC (Vincristine 2mg/m^2/dose,day1; Doxorubicin 37.5 mg/m^2/d, day 1 and 2; Cyclophosphamide 1200 mg/m^2,day1) and IE regimen (Ifosfamide 1800 mg/m^2/day x 5 days and Etoposide 100 mg/m^2/day x 5 days) with G-CSF support (5 mcg/kg/d x 6 days). Interval debulking surgery was planned at week 12 from start of chemotherapy as local control.

During interval debulking, the mass was seen to be arising from one of the ovaries. Subsequently, oophorectomy was performed and omental deposits were biopsied. Grossly, the specimen showed a tumor measuring 10x6.5x5.5 cm. Outer surface was bosselated with presence of few areas of haemorrhage. Cut surface revealed solid (90%) and cystic (10%) areas. Solid areas had a lobulated appearance with grey-white cut surface and areas of necrosis (Figure 2). Microscopic examination of the representative sections revealed a tumor with cells arranged in sheets, lobules and focal follicle-like spaces. The cellular morphology was similar to that seen on FNAC smears. Large areas of necrosis were identified (Figure 3A-C). Omental deposits were confirmed on microscopy (Figure 3D). On performing immunohistochemistry, the tumor cells showed positivity for pan-cytokeratin, vimentin, CD99 (dot-like), WT1 and showed retained nuclear expression for INI1 (Figure 3E-H). FLI1 showed patchy positivity. They were negative for chromogranin and inhibin (Figure 3I). There was loss of expression of SMARCA4/BRG1 protein in the tumor cells. In view of the overall clinical, cytomorphological and immunohistochemical features, a final diagnosis of

small cell carcinoma of ovary, hypercalcemic type was rendered. Patient was started on chemotherapy, however, she succumbed to the disease, 7 months after her initial presentation.

Discussion

Small cell carcinoma of ovary hypercalcemic type (SCCOHT) in an aggressive malignancy of ovary with a 5-year-overall survival rate of 10%, first described by Dickersin et al. in 1982.[2,3] It commonly affects women younger than 40 years of age.[4] However, its occurrence in children is very rare with only a few case reports documenting the same. The youngest reported case is of a 14-month-old child.[5]

Pathogenesis involves sporadic/germline mutations in *SMARCA4/BRG1* and/or *SMARCA2/BRM*. Rarely, familial association has been reported. Witkowski *et al*, reported 2 families, both with 2 females diagnosed with SCCOHT and carrying germline mutations in *SMARCA4/BRG1*.[6] A few of these cases may show mutations in *SMARCB1/INI1*, which are otherwise more commonly associated with malignant rhabdoid tumors elsewhere in the body.

Clinically, the disease has non-specific presentations, most common being abdominal pain and swelling. Two thirds of these patients have associated hypercalcemia.[1] However, symptoms of hypercalcemia like polyuria, polydipsia, constipation, muscle weakness and bone pains; have only been reported in less than 5% of these patients.[7] The hypercalcemia associated with SCCOHT is known to regress following complete surgical excision and re-appear in cases with recurrence and hence can be used as a potential tumor marker for early detection of recurrence in such cases.[2,3,8] Serum calcium levels had not been estimated in the index patient, as a diagnosis of SCCOHT/MRT was not being suspected and she was being managed initially on the lines of Ewing family of tumors. Radiological features are often non-specific and similar to those seen in other ovarian malignancies. Majority of these patients present with a unilateral, solid, large ovarian mass. Bilateral ovarian involvement is rare and

has been rarely reported in relatives of index cases.[2,8] Owing to the highly aggressive nature of the tumor, approximately half the patients have extraovarian spread at diagnosis.[2] The histopathological features include tumor cells in diffuse sheets with interspersed follicle-like spaces. The tumor cells are typically small round cells with hyperchromatic nuclei and scant to moderate amount of cytoplasm. Approximately 50% of the cases exhibit large cell morphology with nests and/or sheets of large cells with characteristic rhabdoid morphology.[1] Based on the genetic as well as the cytomorphologic resemblance of SCCOHT with malignant rhabdoid tumors elsewhere in the body, it is often referred to as malignat rhabdoid tumor of the ovary. Intranuclear inclusions and intracytoplasmic hyaline globules have also been reported.[8]

SCCOHT is an aggressive tumor with a poor prognosis.[2,9] It usually presents at a higher stage with a 50% one year survival rate. Moreover, treatment strategies are not well standardized, owing to the rarity of the disease. However, most patients undergo surgical resection followed by chemotherapy, with neoadjuvant chemotherapy being given to reduce the bulk of the disease in selected cases.[1,2] The outcome is dismal for patients with extra-ovarian disease, where the disease proves fatal within 2 years.[4] Features associated with favourable prognosis in stage IA tumors include normal calcium levels at diagnosis, age more than 30 years, tumor size less than 10 cm and absence of large rhabdoid cells microscopically.[2] Several chemotherapy protocols have been used, with variable success rates, including those used in the treatment of malignant surface epithelial ovarian tumors and malignant germ cell tumors. Commonly employed regimens include, (a) cyclophosphamide, doxorubicin with or without cisplatin, and hexamethylmelamine; (b) paclitaxel in combination with cisplatin for advanced stages; (c) cisplatin, vinblastine, or bleomycin with etoposide regimens.[10-12] Radiation has been rarely used for such patients as is evidenced by

few previous reports, however, some of these studies have claimed better survival with radiotherapy.[2,3,13]

Only a few studies have reported the cytological features of SCCOHT, majority of which have described the intra-operative findings and cytology in body fluids.[7,8,14-18] Trichia *et al*, reported the cytologic features as seen on touch imprints prepared at the time of frozen section.[8] They described epithelial cells which were polygonal with round nuclei, mild to moderate nuclear atypia, indistinct nucleoli and moderate amount of vacuolated or eosinophilic cytoplasm.[8] To the best of our knowledge, ours is the first report to describe the cytological features of SCCOHT on fine needle aspiration cytology. Morphologic differential diagnoses commonly include granulosa cell tumor, small cell carcinoma ovary, pulmonary type (SCCOPT), dysgerminoma, non-Hodgkin's lymphoma and undifferentiated carcinoma. Some of the important features that can help in differentiating these tumors from SCCOHT have been listed in table 1.[8,9,14-18]

To conclude, SCCOHT is a rare malignancy, predominantly affecting younger women. It carries a poor prognosis and management requires prompt diagnosis followed by aggressive multimodality treatment. A high index of clinical suspicion, especially in young women with abdomino-pelvic masses and hypercalcemia, and knowledge of its characteristic cytologic, histopathologic and immunohistochemical features can help in timely diagnosis and appropriate treatment, which might help in improving the survial in such patients.

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Table 1: Histopathologic mimics of small cell carcinoma of ovary, hypercalcemic type and their characteristic features

Tumor	Age range	Gross appearance	Architecture	Cell characteristics	Immunohistochemistry
SCCOHT	Less than 40 years	Large predominantly solid tumors. Necrosis, haemorrhage and cystic degeneration- common	Diffuse sheets with interspersed follicle-like spaces	Small round cells with hyperchromatic nuclei and scant amount of cytoplasm	WT1, CK, EMA, CD10, calretinin-positive; Inhibin-negative
Juvenile Granulosa cell tumor	Mostly children; wide age range	Solid- cystic with tan to yellow appearance.	Diffuse sheets, insular pattern, Call-Exner bodies	Uniform pale round nuclei with nuclear grooves and scant pale cytoplasm	Inhibin, calretinin, FOXL2, WT1, CD56- positive; CK7, EMA-negative
SCCOPT	Postmenopausal	Large predominantly solid tumors with frequent necrosis.	Diffuse sheets. Most have a component of surface epithelial tumor.	Small round cells with moulding, salt and pepper chromatin and scant cytoplasm	Chromogranin, synaptophysin, CD56- variable positivity; CK- +/-
Dysgerminoma	Children and young women	Solid, fleshy tan or white cut surface. Haemorrhage, necrosis, cystic degeneration- may be present	Sheets and nests, interspersed by fibrous septae containing lymphocytes	Medium sized nuclei with vesicular chromatin, prominent nucleoli, abundant eosinophilic to clear cytoplasm and distinct cell borders	PLAP, C117, D2-40, OCT-4, SALL4-positive; EMA- negative
NHL	Wide age range	Solid, fleshy to firm and rubbery. Haemorrhage, necrosis, cystic degeneration- in minority	Diffuse sheets (architecture varies depending on type of lymphoma)	Medium to large sized, coarse chromatin, conspicuous nucleoli and scant amount of cytoplasm	CD45- positive; WT1, CK, EMA, CD117-negative

*SCCOHT: small cell carcinoma of ovary, hypercalcemic type; SCCOPT: small cell carcinoma of ovary, pulmonary type; NHL: non-Hodgkin's lymphoma

Figure Legends

Figure 1: A-C: Smears from the pelvic mass showing tumor cells in clusters and scattered singly, with moderate nuclear pleomorphism, coarse clumped chromatin and moderate amount of cytoplasm (May Grunwald Giemsa; A: 10x; B: 20x; C: H&E, 20x); D-F: Sections from cell block showing areas of necrosis and similar morphology of tumor cells with some cells having rhabdoid appearance (H&E; D:4x, E:40x, F:40x); G-I: Immunocytochemistry on cell-block showing tumor cells to be positive for vimentin (G: 10x), para-nuclear dot-like positivity for CD99 (H: 20x) and negative for myogenin (I: 20x)

Figure 2: A, B: Gross specimen showing the tumor with bosselated outer surface and solid-cystic cut surface. Cut surface is lobulated with greyish-white solid areas, areas of necrosis and haemorrhage

Figure 3: A-C: Sections from ovarian mass showing tumor arranged in sheets, lobules and follicle-like spaces with intervening areas of necrosis. Tumor cells have similar morphology as seen on the aspirate smears (H&E; A: 4x; B: 20x); C: Section showing omental deposits (H&E; 4x). D-I: Immunohistochemistry showing tumor cells to be positive for CK (D: 20x), Vimentin (E: 20x), WT1 (F: 20x) with retained INI1 expression (G: 20x) and loss of nuclear expression of BRG1 (H:20x) and negative for chromogranin (I: 20x)