An unusual case of clear cell sarcoma presenting as multiple abdominal masses confirmed by RT-PCR

Dear Editor,

Clear cell sarcoma (CCS) is a rare tumor accounting for 1% of all soft tissue sarcomas typically involving the distal extremities. Though it shares histological and immunohistochemical overlap with malignant melanoma, it is considered as a separate entity characterized by EWS-ATF1 fusion transcript.[1] It has a rapid and fatal progression with frequent metastases to regional lymph nodes and lungs. Mesenteric dissemination and its initial presentation as metastasis are extremely rare.

A 43-year old male albino since birth presented with intermittent dull aching abdominal pain of one month duration and constipation for 10 days to an outside hospital. Subsequent imaging revealed multiple mesenteric masses for which laparoscopic biopsy was performed and diagnosed as gastrointestinal stromal tumor (GIST) solely based on morphology without any immunohistochemistry (IHC). He was started on Imatinib; as the symptoms persisted even after 7 months of treatment he was referred to our institute for further management. On physical examination, a hard mobile lump was noted in the right iliac fossa. Laboratory investigations and biochemical parameters were within normal limits. Contrast-enhanced computed tomography (CECT) abdomen showed well-defined homogenously enhancing confluent and discrete soft tissue nodular lesions in the mesentery, right lumbar region, and left perinephric space, largest measuring 5.7 × 3 cm [Figure 1]. There was circumferential wall thickening of the small bowel loop. Bilateral iliac and inguinal lymph nodes were also noted. Exploratory laparotomy and repeat biopsy was done.

Microscopic findings

Histologically, the tumor cells were arranged in lobules separated by thin fibrous septae with peripheral rim of lymphocytes. Cells were polygonal with clear to eosinophilic cytoplasm and round to oval vesicular nuclei with prominent nucleoli [Figure 2a-c]. The IHC panel performed included CD 117, DOG 1, SMA, S100, CD34, HMB45, vimentin, PanCK, chromogranin, and Ki-67 [Figures 2d-i and Figure 3]. The IHC results are summarized in Table 1. These histological and IHC features were consistent with the diagnosis of clear cell sarcoma.

Further clinical and imaging studies did not reveal any primary. The patient was referred to the department of oncology for chemotherapy. He was started on ifosfamide- and adriamycin-based regimen. Ifosfamide and adriamycin were given at doses of 1.5 and 50 mg/m^2. It was planned to give total of six cycles of chemotherapy.

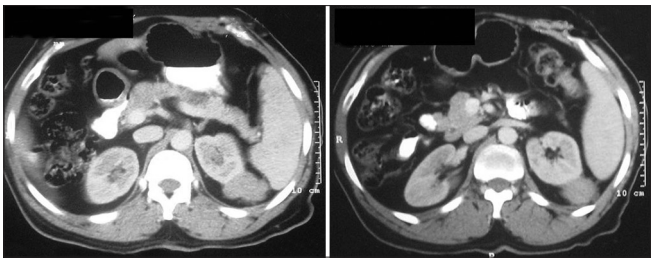

Figure 1: CECT abdomen showing well-defined homogenously enhancing confluent and discrete soft tissue nodular lesions in the mesentery and left perinephric space

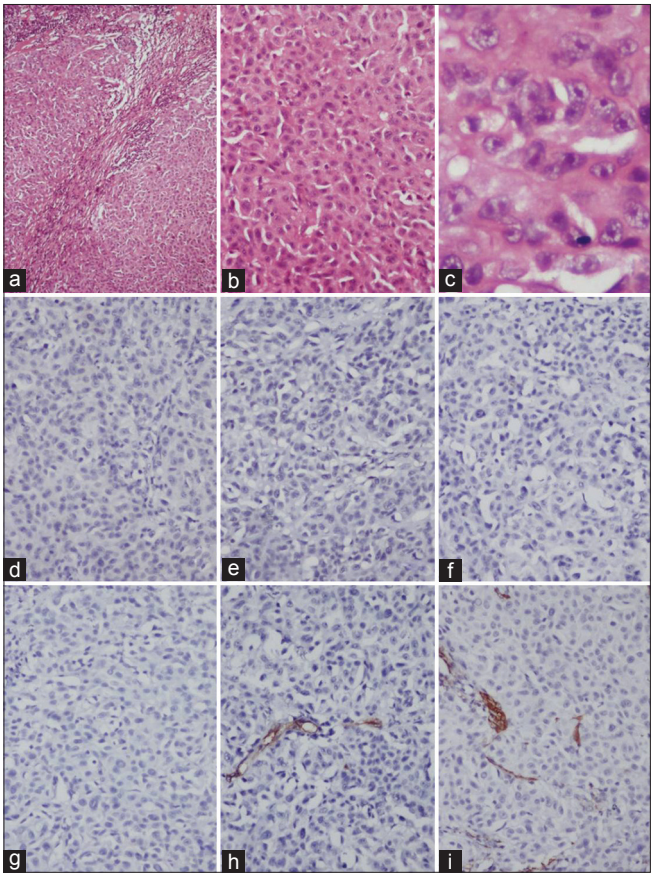

Figure 2: Microscopic findings of mesenteric mass. (a) lobules of tumor cells separated by fibrous septa (H and E; X40); (b) tumor cells arranged in sheet (H and E; X100); (c) tumor cells showing round to oval vesicular nucleus with prominent nucleoli and acidophilic cytoplasm (H and E; X400); negative IHC staining of tumor cells with (d) CD117; (e) DOG1; (f) PanCK; (g) chromogranin; (h) CD34; (i) SMA (HRP-Polymer; X100)

Table 1: Results of immunohistochemistry

IHC marker	Results
CD117	Negative
DOG1	Negative
CD34	Negative
SMA	Negative
S100	Positive
HMB45	Positive
Vimentin	Positive
PanCK	Negative
Chromogranin	Negative
Ki67	16%

IHC=Immunohistochemistry

Patient had progressive disease after three cycles of chemotherapy. He developed hemiplegia and additional swelling over the left thigh. Aspiration smears from the thigh swelling were cellular and showed clusters and singly scattered polygonal to spindle cells. These cells had round to oval vesicular nuclei, prominent nucleoli, and moderate to abundant cytoplasm. The cell block sections also showed similar cells. IHC was done on the cell block sections and the cells were also positive for HMB45 and S100 supporting the diagnosis of recurrent CCS [Figure 4]. Further molecular analysis with RT-PCR was performed using formalin fixed paraffin embedded tissue.

RT-PCR methodology

To detect and determine the type of EWS-ATF1 and EWS-CREB1 chimeric transcript in formalin-fixed paraffin embedded tissue, a reverse transcriptase polymerase chain reaction (RT-PCR) assay was set up. Deparaffinization and total RNA extraction was performed using the Qiagen RN easy extraction kit for FFPE as directed by the manufacturer with the following modifications: three xylene washes followed by three 100% ethanol washes. The total RNA was reverse transcribed in to cDNA using the Fermentas Revert Aid H Minus first strand cDNA synthesis kit as per the manufacturer's protocol. The integrity of the RNA was evaluated by running a parallel PCR for a 247 bp fragment of the ubiquitously expressed PGK gene. For RT PCR detection of EWS –ATF1/CREB 1 fusion transcript, we used the EWS ex7 F1 forward primer with the CREB1 ex7 – REVC primer (specific for CREB1; sequence GTACCCCATCGGTACCATTGT) and the consensus CREB1 ex7 – REVA primer (binds both CREB1 and ATF1; sequence: TCCATCAGTGGTCTGTGCATACTG) PCR was performed for 35 cycles at 60°C. Amplicons were visualized on a 2% agarose gel using ethidium bromide staining.

This specific case showed a distinct and specific product at the expected band range with the EWS ex7 F1 forward primer and the consensus CREB1 ex7 – REVA primer (binds both CREB1 and ATF1), while no product was seen with EWS ex7 F1 forward primer and CREB1

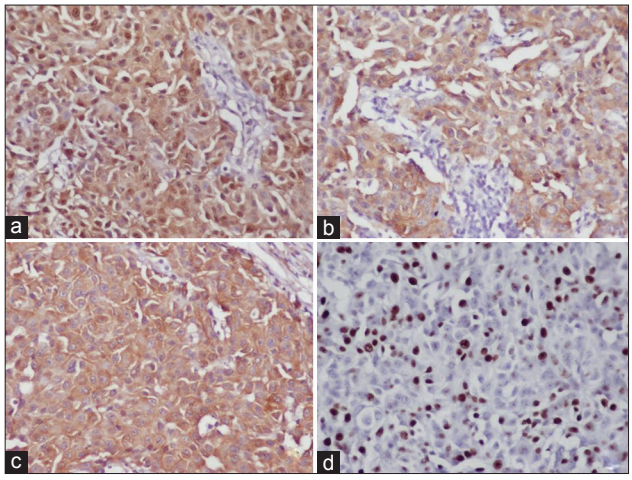

Figure 3: Positive staining of tumor cells on IHC with (a) S100, (b) HMB45, (c) vimentin, (d) Ki67(LI -16%) (HRP-Polymer; X100)

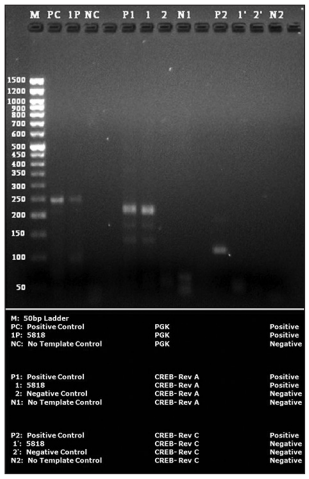

Figure 5: Results of PGK-CREB1 assay

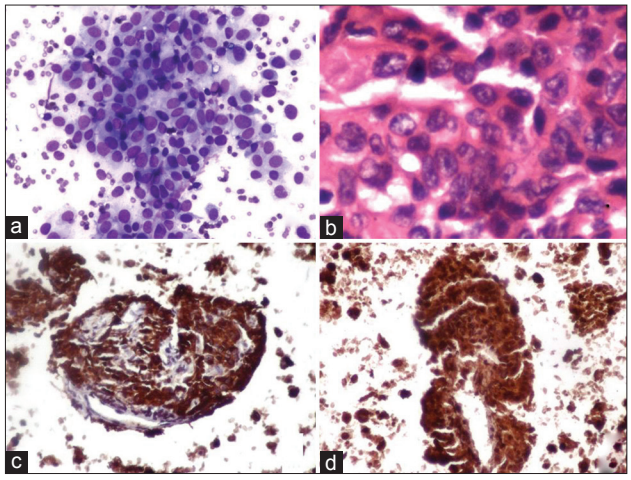

Figure 4: (a) Fine needle aspiration smears of thigh mass showing cluster of polygonal tumor cells with round to oval nuclei and moderate cytoplasm (MGG; X100). (b) Cell block section showing tumor cells with similar morphology (H and E; x400). (c) and (d) IHC with HMB45 and S100 done on cell block sections showing positive staining (HRP-Polymer; X100)

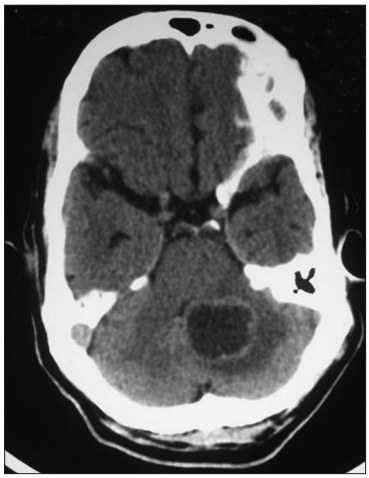

Figure 6: CECT brain showed a hypodense lesion with peripheral enhancement in the left cerebellar hemisphere

ex7 – REVC primer (specific for CREB1), thus, confirming the presence of EWS-ATF1 chimeric fusion transcript in this case [Figure 5].

Further CECT brain showed a 3 × 3.2 cm hypodense lesion with peripheral enhancement in the left cerebellar hemisphere suggestive of metastases [Figure 6]. He received palliative radiotherapy to brain (3 gray/fraction/day × 10 fractions). He was explained the nature of his disease and poor prognosis due to progression while on therapy. He opted for best supportive care.

Clear cell sarcoma was first described by Franz M Enzinger in 1965. It preferentially affects adolescents and young adults. It has a predilection for deep soft tissues of lower extremities in close proximity to tendons and aponeuroses. The most frequent sites of involvement are foot and ankle followed by knee, thigh, hand, forearm, elbow, and shoulder in descending order of frequency.[2]

Primary gastrointestinal CCS is also described that is rare and involves small intestine, large intestine, and stomach.[3] The first visceral case was described in the duodenum in 1993. In 1998, first case of multiorgan localization was reported in a 64-year-old man involving stomach, pancreas, mesocolon, left thigh, and left axilla.[4]

CCS has a high propensity of local recurrence, regional lymph node, and distant metastasis. There are several lines of evidence supporting melanocytic differentiation. Like melanomas, CCS shows a propensity for regional lymph node metastasis but lacks typical melanoma-like diffuse pattern of distant metastatic spread. Majority of CCS show immunoreactivity for melanoma markers like HMB45 and S100. They also demonstrate melanosomes ultrastructurally. Despite these similarities, CCS represents a unique entity characterized by a distinct and recurrent chromosomal translocation t (12; 22) (q13, q12) leading to fusion of ATF1 gene on 12q13 to the EWSR1 gene at 22q12 in 90% cases. This translocation can be demonstrated by conventional cytogenetics, reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescent *in-situ* hybridization (FISH). EWS/ATF1 binds to Micropthalmia transcription factor (MITF) leading to melanocytic differentiation. Recent reports have shown a high incidence of BRAF mutations in malignant melanoma while it is rare in CCS. Hocar *et al.* demonstrated mutation in BRAF and NRAS in the two of twenty two CCS. However, these tumors also showed ATF1-EWS fusion gene and were considered atypical.[5]

The expression of MITF-M isoform is required for differentiation of melanocytes. A direct link between EWS-ATF1 and MITF expression in clear cell sarcoma has been demonstrated recently.[6] EWS-ATF1 deregulates MITF expression by directly binding to the cyclic AMP response element located in the melanocyte-specific MITF promoter resulting in albinism from defective pigment production within viable melanocytes. Inhibition of MITF activity in clear cell sarcoma decreases both pigmentation and the expression of markers of melanocytic differentiation previously demonstrated to be MITF targets. While no drug currently exists to directly suppress MITF, the identification of new targeted therapies to block MITF is under research.

Primary CCS of gastrointestinal tract uniformly expresses S100 but lacks melanocytic differentiation being negative for HMB45. These are characterized by presence of EWS-CREB1 fusion transcripts in majority of cases.[7] Melanocytic differentiation evidenced by HMB 45 positivity and the presence of EWS –ATF1 fusion transcript on RT-PCR favors a diagnosis of peripheral CCS over gastrointestinal CCS in the present case.

According to the literature, CCS most commonly metastasizes to lymph nodes and lungs, and less frequently to skin, bones, liver, heart, and brain.[2] A rare case CCS of neck metastasizing to breast 11 months after surgery for primary lesion and another case of CCS presenting with capitate bone metastasis has been reported.[8,9] Sara *et al*. in their series of 17 cases of CCS have documented three cases presenting with metastases at initial diagnosis. These include one of case regional lymph node metastases and another case with distant metastases involving lungs and liver. The third case showed both regional metastasis to lymph nodes and distant lung metastases.[10] About 60-70% of patients with CCS develop metastases at a mean time interval of 18 months to 6 years. In the series of Churg and Enzinger, the average time between diagnosis and recurrence was 2.6 years and between diagnosis and metastasis 3.5 years.[2]

The primary gastrointestinal CCS frequently presents with regional lymph node metastases and mesenteric dissemination is noted in about half of the patients at the time of diagnosis. Most of these patients also presents with liver, peritoneal, pancreatic, and lung metastasis.[3] Peripheral CCS presenting with mesenteric dissemination at initial presentation as noted in present has not been described earlier.

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