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Case Report

Pathology Section

Cytodiagnosis of Extraskeletal Ewing's Sarcoma and its Confirmation by Fluorescence in situ Hybridization

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ABSTRACT

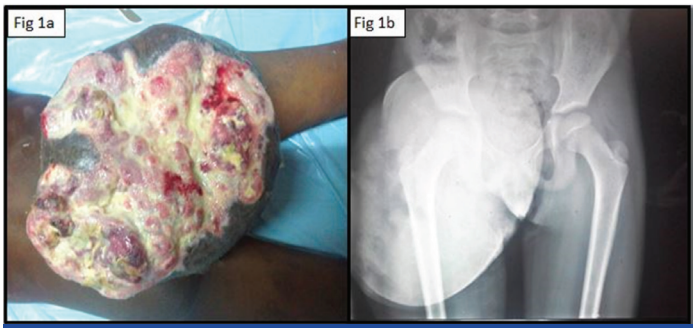
Extraskeletal Ewing's sarcoma is an aggressive malignant small round cell tumour usually occuring in children and adolescents. It needs to be differentiated from other malignant small round cell tumours and immunohistochemistry plays a pivotal role in establishing the diagnosis. Fluorescence in situ hybridization or real time-polymerase chain reaction helps in confirming the diagnosis by demonstration of EWS-FLI1 translocation, which is found in approximately 85% of the cases. We report a case of extraskeletal Ewing's sarcoma in a10-year-old male, who presented with a right gluteal region mass. Fine needle aspiration and cell block preparation followed by a panel of immunohistochemical markers were performed. Immunohistochemistry for CD99 and FLI1 was positive. EWS-FLI1 translocation was confirmed by fluorescence in situ hybridization.

Keywords: Cell block, FLI1, FNAC

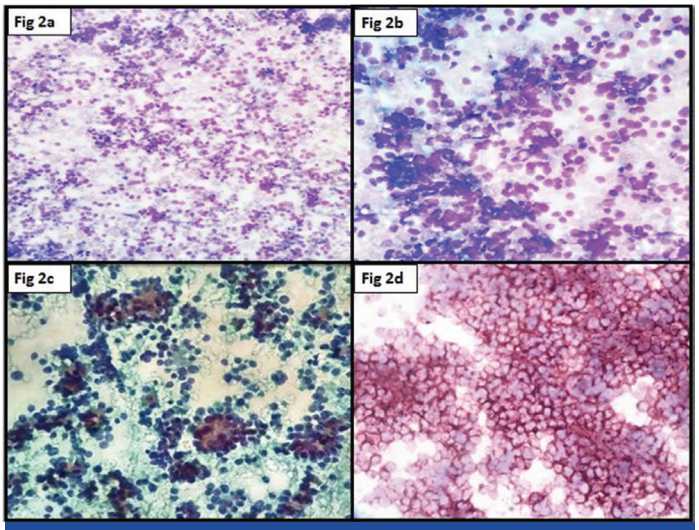
CASE REPORT

A 10-year-old male presented with a right gluteal region mass since a duration of six months. The patient also complained of loss of appetite and dyspnoea. On examination, there was a right gluteal ulcerated mass measuring 10× 8×8 cm [Table/Fig-1a]. There was no lymphadenopathy or organomegaly.

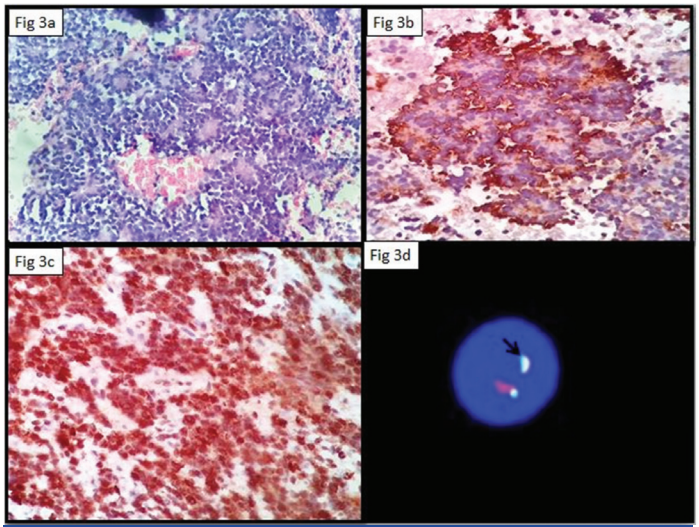
X-ray of the right pelvic region revealed a soft tissue mass measuring 21x10 cm, not involving the underlying bone [Table/Fig-1b]. Contrast enhanced computed tomography showed an exophytic heterogenous soft tissue mass measuring 21.8x10 cm arising from right gluteus maximus muscle. FNAC was performed and smears were stained for May-Grunwald Giemsa (MGG) and Papanicolaou (Pap) stains, and material was collected for cell block preparation. Cytology smears were cellular and showed dispersed, small, monomorphic round cells with fine nuclear chromatin, round nuclei and scanty clear cytoplasm [Table/Fig-2a,b]. Many cells showed irregularly vacuolated cytoplasm. Occasional rosette formation was also seen [Table/Fig-2c]. Immunocytochemistry showed tumour cells positive for CD99 and negative for desmin, Leucocyte Common Antigen (LCA) and TdT [Table/Fig-2d]. Based on these cytological and IHC features, a diagnosis of extraskeletal Ewing's sarcoma was offered. The cell block sections showed sheets of small round cells with rosette formation [Table/Fig-3a]. Immunohistochemistry (IHC) markers were performed on cell block, which showed membranous positivity for CD99 and nuclear positivity for Friend leukaemia integration-1 (FLI1) [Table/Fig-3b,c]. The tumour cells were negative for Pancytokeratin, Bcl2, desmin, MyoD1, LCA, TdT, chromogranin, Neuron specific enolase (NSE)



[Table/Fig-1]: (a) Right gluteal ulcerated mass. (b) X-ray of the right pelvic region revealed a soft tissue mass without any involvement of the underlying bone.



[Table/Fig-2]: (a&b) Cytology smears were cellular showing dispersed, small, monomorphic round cells with fine nuclear chromatin and round nuclei and scanty clear cytoplasm (MGG, 4x and 40x). (c) Rosette formation in cytology smear (Pap, 40x). (d) Strong membranous positivity for CD99 in the cells (ICC, 40x).



[Table/Fig-3]: (a) Cell block section showed sheets of small round cells with rosette formation (H&E, 10x). (b) Cell block showing strong membranous positivity for CD99 (DAB, 400x). (c) Table/Fig 3c: Cell block showing strong nuclear positivity for FLI1 in the tumour cells (DAB, 40x) (d) FISH showing positive fusion for EWS-FLI1 with one yellow fusion signal in the nuclei (Arrow)

and S100. Thus a wider panel of IHC markers performed on cell block helped in confirming the diagnosis. A trucut biopsy was done. On histopathological examination, the tumour was confirmed as extraskeletal Ewing's sarcoma. The EWS-FLI1 gene fusion was demonstrated by fluorescent in situ hybridization (FISH) in the cell block tissue [Table/Fig-3d].

During this time, a chest X-ray and bone marrow examination were done as a part of metastatic work-up. Chest X-ray showed multiple nodular lesions in bilateral lungs suggestive of metastasis. Bone marrow examination showed metastatic deposit from the primary tumour.

The patient was started on chemotherapy with vincristine, doxorubicin, cyclophosphamide (VAC) alternating with etoposide and ifosfamide. Although the patient responded well to chemotherapy, he presented with local relapse and died after one year of initial diagnosis.

DISCUSSION

Ewing's sarcoma family of tumours (ESFTs) represent a family of malignant small round-cell neoplasms, which include Ewing's sarcoma (ES) of bone, primitive neuroectodermal tumour, extraskeletal Ewing's sarcoma (EES) and Askin tumour [1]. ESFTs generally originate in bone tissue, but they can occasionally originate in soft tissue, known as EES, which constitutes 6% to 47% of all ESFTs and 1.1% of all malignant soft tissue tumours [1,2]. EES shares the morphological, IHC & molecular features with its osseous counterpart [1-3].

EES is considered a neoplasm of children and adolescents, and the usual sites are the trunk, extremities, retroperitoneum and head and neck region [1]. However, it has been reported to arise in a variety of anatomical sites and in all age groups [1,4,5].

The differential diagnosis includes other malignant small round cell neoplasms like rhabdomyosarcoma, lymphoma, neuroblastoma, synovial sarcoma, desmoplastic small round cell tumour, extra-skeletal mesenchymal chondrosarcoma [2,4,5]. Considering the large number of differentials, IHC markers and molecular genetic analysis by FISH and real time-polymerase chain reaction (RT-PCR) are pivotal for confirmation of the diagnosis of EES [2,3].

CD99 positivity has been reported in 84% to 100% of ES, however it is not specific for ES [4]. Positive staining for CD99 has been demonstrated in lymphoblastic lymphoma, synovial sarcoma, rhabdomyosarcoma and desmoplastic small round cell tumour [4,5]. ES shows diffuse membranous positivity for CD99 whereas synovial sarcoma shows strong cytoplasmic positivity [5]. The other tumours show focal and weak cytoplasmic positivity for CD99 [5]. FLI1 is a specific marker for ES, however it is variably positive in lymphoblastic lymphoma [6]. In our case, tumour cells showed membranous positivity for CD99 and nuclear positivity for FLI1. We did a panel of negative IHC markers like chromogranin and NSE to rule out neuroblastoma, Desmin and MyoD1 to rule out rhabdomyosarcoma, LCA and TdT to rule out lymphoblastic lymphoma, Pancytokeratin, Desmin and NSE to rule out desmoplastic small round cell tumour, Pancytokeratin and Bcl2 to rule out synovial sarcoma, NSE and S100 to rule out extraskeletal mesenchymal chondrosarcoma.

ES is characterized by balanced translocations between the EWS gene and the ETS family members like FLI1 and EWG [2]. Approximately 85% of cases have the t(11;22)(q24;q12) translocation resulting in the formation of a chimeric gene in which the 5' end of the EWS gene is fused to the 3' end of FLI1 [3]. Exons 1–7 of EWS are fused to exons 6–9 of FLI1 (type 1fusion) in 60% of cases harbouring the EWS-FLI1 translocation and in the remaining cases exons 1–7 of EWS are fused to exons 5–9 of FLI1(type 2 fusion) [2]. Tumours with EWS-FLI1 type 1 fusion transcript have better outcome when compared with tumours with type 2 fusion transcript [5]. The second most common translocation accounting for 10% of ES is t(22;21)(q22;q12) resulting in the formation of EWS-EWG gene [2,3]. Occurrence of fusions between EWS and other ETS family members is less common. They include EWS-ETV1 t(7:22), EWS-ETV4 t(17;22) and EWS-FEV t(2;22). Both RT-PCR and FISH usually provide concordant results [3].

EES has a poorer prognosis as compared to its osseous counterpart with high incidence of local recurrence and distant metastasis [1]. The most common site of metastasis is the lungs [4]. Patients with tumour size more than 8cm, high serum lactate dehydrogenase, metastasis at presentation, poor histological response to chemotherapy and positive surgical margins have a poor prognosis. Multimodality treatment consists of adequate surgical resection, aggressive chemotherapy and radiotherapy [1]. Unresectable cases or with metastasis are treated with chemotherapy benefit of which is more often limited to extending progression-free survival [1]. The present case had a tumour size more than 8cm and had pulmonary and bone marrow metastasis at the time of presentation.

CONCLUSION

EES has an aggressive behaviour. Cytomorphology followed by IHC and confirmation of specific translocation by FISH or RT-PCR has become a reliable mode of diagnosis of EES. Cell block should be prepared as it provides material for ancillary techniques.

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