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CLINICOPATHOLOGICAL AND IMMUNOPHENOTYPIC CHARACTERISTICS OF EWINGS SARCOMA FAMILY OF TUMORS: SPECIAL EMPHASIS ON ROLE OF FRIEND LEUKEMIA INTEGRATION - 1(FLI-1) ANTIBODY AND OCCURRENCE OF TUMOR ON RARE SITES

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ABSTRACT

Objective: Ewings sarcoma /Primitive neuroectodermal tumor (ES/PNET) is one of the aggressive malignant round cell tumors affecting mainly children and adolescents. This study aimed to emphasize the role of FLI-1 antibody along with CD99 in diagnosing ES/PNET and to keep this tumor in differential diagnosis even at some rare sites.

Material and Method: This descriptive and retrospective study was conducted from January 2014 – May 2016 and included 58 patients of ES/PNET. Histopathological and IHC findings were reviewed and the diagnosis of ES/PNET was confirmed. The tumors were further categorized into classic Ewings, PNET and atypical subtypes. Panel of IHC markers included vimentin, CD99, FLI-1, PanCK, LCA, Desmin, Myogenin, NSE, Synaptophysin, S100 and Chromogranin.

Results: Clinical Findings- We studied 58 patients of ES / PNET of whom 63% (37/58) were males, 37% (21/58) were females. The age ranged between 3- 65 years (mean 20 years).

Histological Findings: 57% of cases (33/58) exhibited classic morphology, 40% (23/58) were classified as PNET. There were only two cases of atypical Ewings sarcoma including large cell and Clear cell variant.

Immunohistochemical Findings: Strong membranous CD99 expression was seen in 100%, FLI -1 nuclear staining in 85% , NSE in 50%, S100 in 30%, Synaptophysin in 10% and EMA in 2% (1/58) cases. All other markers included in the panel were negative.

Tumor on Rare Sites: 10 Cases were of primary extradural spinal PNET, 1 intramedullary spinal, 2 brain parenchyma, 2 nose & nasopharynx , and one rare case of urinary bladder.

Conclusion: Clinical details, histomorphology and IHC includingCD99 and monoclonal FLI-1antibodies may help in supporting diagnosis of EFT if cytogenetic and FISH techniques are not available at any centre.

Key Words: Ewings sarcoma, Primitive neuroectodermal tumor, CD99, FLI-1

INTRODUCTION

Ewings sarcoma/Primitive neuroectodermal tumor (ES/PNET) is one of the aggressive malignant small round cell tumors that may arise virtually anywhere but is most common in bone, deep soft tissue of extremities and parenchyma.

This tumor was first reported in 1918 by Arthur Purdy stout

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as ulnar nerve tumor[1]. Three years later in 1921 James Ewing reported a round cell neoplasm calling it a "diffuse endothelioma of bone "in the radius[2].The soft tissue Ewing's sarcoma was first described in 1975 by Angervall and Enzinger[3]. Seemayer and colleagues described Peripheral neuroectodermal tumors (PNET) arising in soft tissues at the same time[4]. In 1979, Askin and coworkers described the malignant small cell tumor of the thoracopulmonary region (Askin tumor) similar to PNET[5].

With the advent of IHC, cytogenetic and molecular techniques it is universally accepted that these tumors shared identical features and these were designated as ES/PNET family of tumors(EFT) [6,7].70-80% of cases show morphology of classic Ewings sarcoma and up to 20% show atypical features including large cell, spindle cell, sclerosing, clear cell, vascular or admantinoma like patterns[8]. Diagnosis of PNET was considered when homer wright rosettes were seen or when any two different neural markers were positive according to criteria proposed by Schmidt et al[9].

A panel of immunomarkers such as vimentin, CD99, FLI-1, Desmin, Myogenin, Leucocyte common antigen (LCA), cytokeratin (CK) ,Neural markers like non specific enolase (NSE), S100,Chromogranin and Synaptophysin are usually used to distinguish various small round cell tumors. CD99(MIC2) has not proven to be specific for Ewings sarcoma family of tumors despite initial promise. Approximately 90% of EFT have a specific t (11; 22) (q24; q12) that results in fusion of the EWS and FLI-1 protein; hence we included both CD99 and FLI-1 in the panel.

Although molecular testing is gold standard but many centers still do not have this facility, hence IHC plays an important role in differentiating these tumors. The aim of our study was to analyse the clinicopathological and immunophenotypic characteristics of 58 cases of Ewing's sarcoma/ primitive neuroectodermal tumors from tertiary care centre in Jaipur, Rajasthan with special emphasis on role of FLI-1 protein, and occurrence of tumors at some rare sites.

Material & Method: This descriptive and retrospective study was conducted from January 2014 – May 2016 and included 58 patients of ES/PNET. Clinical details were collected from the case files. Clinical features such as age, sex, site of involvement, radiological findings, and soft tissue extension were evaluated.

The paraffin blocks of all cases were retrieved. Histopathological and immunohistochemical (IHC) findings were reviewed and the diagnosis of ES/PNET was confirmed. The tumors were further categorized into classic Ewings, PNET and atypical subtypes.

Diagnosis of PNET was considered when Homer Wright or Flexner Wintersteiner rosettes were seen or when any two different neural markers were positive. Atypical Ewings sarcoma was labeled when cells were large pleomorphic with coarse chromatin, prominent nucleoli and absent rosettes.

Panel of IHC markers included vimentin, CD99, FLI-1, CK, LCA, Desmin, Myogenin, NSE, Synoptophysin, S100, and Chromogranin. Additional IHC markers like glial fibrillary acidic protein (GFAP), INI-1, terminal deoxyneotidyl transferase (Tdt) etc. were also included according to site of lesion and differential diagnosis.

IHC analyses for all above markers were done on 4µ thick section taken on tissue bond coated slides by immunoperoxidase method with respective antibodies as per manufactures instructions. Antigen retrieval was done by the heat induced epitope retrieval method in diva decloaker chamber by using citrate buffer (PH 6.0) after tris buffer wash. Endogenous peroxidase was blocked by hydrogen peroxide solution. Sections were incubated with primary antibody for 1 h 30 min. Biocare's HRP polymer is applied as secondary antibody and 3'3 diaminobenzidine as chromogen substrate.

Appropriate positive and negative controls were included.

RESULTS

Clinical details

This retrospective and descriptive study included 58 patients of ES / PNET of whom 63% (37/58) were male, 37 %(21/58) were female. The age ranged between 3- 65 years (mean age 20 years).

Most common site of involvement was skeletal in 61% (35/58), followed by soft tissue in 29% (17/58) and parenchyma of rare sites in 10% (6/58) cases. Rare sites included 10 cases of primary extradural spinal PNET, 1 case of intramedullary spinal, 2 cases of brain parenchyma, one case of nose, nasopharynx and urinary bladder each (Table 1).

The most common bones involved were of upper & lower extremities followed by iliosacral region. The patients presented with local swelling as most common complaint followed by pain, fever, weakness of limbs and pathological fracture.

Localized disease at presentation was seen in 29% (17/58) cases whereas locally advanced disease was seen in 15% (9/58) cases. Soft tissue extension was noted in 74 % (20/27) of osseous cases.

Follow up related to recurrence, metastasis and treatment history was available in very few patients hence not included in this study.

Histological Characteristics

57% of cases (33/58) exhibited classic morphology (Figure1). Microscopically tumor shows solidly packed, lobular pattern of strikingly uniform round cells with high N/C ratio. The individual cells have a round to ovoid nucleus with a distinct nuclear membrane, fine chromatin and 2-3 inconspicious nucleoli. The cytoplasm was ill defined scanty, pale, clear and sometime vacuolated. Neoplastic cells were positive for PAS and digested with D-PAS indicating accumulation of glycogen.

Some of the cases showed darker crushed spindled cells. The number of mitotic figures varied from 1 per 10 HPF to 7 per 10 HPF. Most of the cases showed necrosis.

40% (23/58) cases were classified as PNET characterized by presence of rosettes (figure 2). Most of the rosettes contain a central solid core of neurofibrillary material (Homer Wright rosettes) rarely rosettes contain a central lumen or vesicle (Flexner Wintersteiner rosettes).

There were only two cases of atypical Ewings sarcoma in the present study showing large cell and clear cell variant. Large cell variant was seen in nasopharynx and clear cell variant was noted in spinal cord. In large cell variant, cells were large, pleomorphic with coarse chromatin, prominent nucleoli and absent rosettes (figure 3).Large areas of necrosis were also seen. In clear cell variant, tumor cells had abundant clear cytoplasm with small round cell morphology (figure 4).

Immunohistochemical Findings

All of the cases (58/58) studied showed strong membranous CD99 expression (figure 5) and cytoplasmic Vimentin.

FLI -1 nuclear staining was noticed in 85% cases (49/58) (Figure 6).

Immunostaning for NSE was seen in 50% cases, S100 in 30% cases and Synaptophysin in 10% cases. EMA was focally positive in clear cell variant (Figure 7).

All other markers included in the panel were negative (table 2).

DISCUSSION

This series comprised patients of Ewings sarcoma / PNET of all ages who were enrolled and treated in a tertiary care center, Jaipur, Rajasthan.

There was male preponderance and the mean age was 20 years. The most common sites of involvement were bone of both upper and lower extremities. The patients presented most often with swelling followed by pain, some have fever and limbs weakness which is in accordance with other studies[10].

Histomorphogically the incidence of classic type was the most common followed by PNET and atypical variants, which is similar to a study done by Folpe et al[11]. In our study we had only 2 cases of atypical Ewing's sarcoma which is quite less as compared to one study[12]and quite similar to other study done by Priya D et al. in which they found 4 cases of atypical Ewing's sarcoma out of total 51 cases of EFT studied[13].

These entire EFT show strong membranous CD99 Positivity. The differential diagnosis that could be considered for Ewings sarcoma with CD99 positivity included lymphoblastic lymphoma, poorly differentiated synovial sarcoma, small cell osteosarcoma, rhabdomyosacoma, desmoplastic small round cell tumor (DSRCT) small cell carcinoma and merkel cell carcinoma[14]. As CD-99 has not proven to be specific marker for EFT despite initial promise, we included other quite specific marker for EFT that is FLI-1. FLI-1 was positive in 85% cases including atypical Ewings sarcoma. Nuclear staining was considered positive with positive endothelial cell nuclei and a subset of lymphocytes serving as internal control.

Results are similar to study done by Llombart bosch et al in 2001, they found FLI-1 expression in 84% (16/19) cases of Ewings sarcoma family of tumors out of 48 studied cases of small round cell tumors[15].

Our results are slightly higher to study done by Folpe AL in 2000[16].He studied 132 cases of CD99 positive mimics of Ewing's sarcoma/ primitive neuroectodermal tumors including 41 cases of Ewings sarcoma. He found FLI-1 positivity in 71% cases of EFT (29/41) and 88% cases of lymphoblastic lymphoma (7/8), rest all were found to be negative.

Previous study done on FLI-1 was by using polyclonal antibody. Rossi et al[17]used monoclonal antibody and found 100% positivity for FLI-1 in EFT cases(15/15).We also used polyclonal antibody that is why positivity rates were lower in our study.

NSE was expressed in50% and S100 in 30% which was similar to previous study done by Kavalar et al[18], where NSE and S-100 were expressed in 66.6% and 25.4% cases respectively.

We had 10 cases of primary intraspinal PNET at our centre .All of our cases were extradural. A case study done by Sundar Venkataraman found that only 19 cases of primary intraspinal PNETs have been reported to date[19].To best of our knowledge we had half of this data at our centre within such a short time duration.

One interesting case at this location was atypical Ewings

sarcoma with ciear cell type showing focal EMA positivity. EMA positivity highlights the epithelial differentiation in the EFT. Previously many studies were done on expression of broad spectrum of AE1/AE3 antibody showing positivity rates ranging from 20% to 40% [20,21]. Machado et al in 2011 studied 415 genetically confirmed ESFT, for expression of CK, EMA and CEA. They found 19.2 %, 6.6% and 20.8% positive cases for these antibodies respectively[22].

We had 2 cases of primary intracranial peripheral PNET (pPNET). The IHC panel included GFAP, EMA, CD-99, FLI-1, CD-34, synaptophysin, INI-1, NSE and MIB-1 score. The cases were positive for CD-99, FL-1and NSE. MIB-1 labeling index was 6% and 45% respectively (Figure 8). PNET at any site show variable but generally high MIB-1 score varying from 0-85%.[23] These cases are different from CNS PNET in the clinical behavior, treatment, prognosis, IHC and molecular genetic study.[24]

We had one case of primary intramedullary spinal PNET in 12 year old male child at D11-L2 label. Bone scan and MRI brain were done to know any other site of primary tumor. Both investigations did not reveal any evidence of tumor elsewhere and a diagnosis of primary intramedullary spinal PNET was made. The diagnosis of such a tumor is very crucial as management strategies are relatively unclear and have a poorer outcome as compared to other primary intramedullary spinal tumors[25].

We had one another interesting case of primary PNET of urinary bladder in 31 year old female patient. These are extremely rare and to our knowledge only 12 cases have been reported so far in the English literature[26].

We had other two interesting cases of nasal cavity and nasopharynx in40 and 10 year old male patients respectively. It is very rare in head and neck region and accounts for only 2-3% of all the cases[27].

CONCLUSION

Clinical details, histomorphology and IHC including CD99 and monoclonal FLI-1antibodies may help in supporting the diagnosis of EFT even in absence of cytogenetic and FISH techniques. EFT should be kept in differential diagnosis of all malignant small round cell tumours even at rare sites.

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Table 1: Clinicopathological characteristics and IHC findings of 6 cases of EFT involving parenchyma

S. No.	Year of diagnosis	Age (year)	Sex	Site of lesion	Histomorphological type	IHC findings (only positive)
1	2015	12	M	Intramedullary mass, D11-L2	PNET absent rosettes	CD99+,FLI-1+,NSE+,S-100+
2	2014	22	F	Parieto-occipital SOL	PNET with rosettes	CD99+,FLI-1+,NSE+
3	2015	15	M	Fronto-temporal SOL	PNET with rosettes	CD99+,FLI-1+,NSE+
4	2014	10	M	Nasopharyngeal mass	Atypical Ewings large cell type	CD99+,FLI-1+
5	2015	40	M	Nasal mass	Classical Ewings	CD99+,FLI-1+
6	2014	31	F	Urinary bladder	Classical Ewings	CD99+,FLI-1+

Table 2: Immunohistochemical findings.

S. No.	Marker	No. of positive cases	Percentage (%)
1	Vimentin	58	100%
2	Panck	Nil	0
3	EMA	1	2%
3	CD99	58	100%
4	FLI-1	49	85%
5	LCA	Nil	0
6	Desmin	Nil	0
7	Mygogenin	Nil	0
8	NSE	29	50%
9	S-100	17	30%
10	Synaptophysin	6	10%
11	Chromogranin	Nil	0

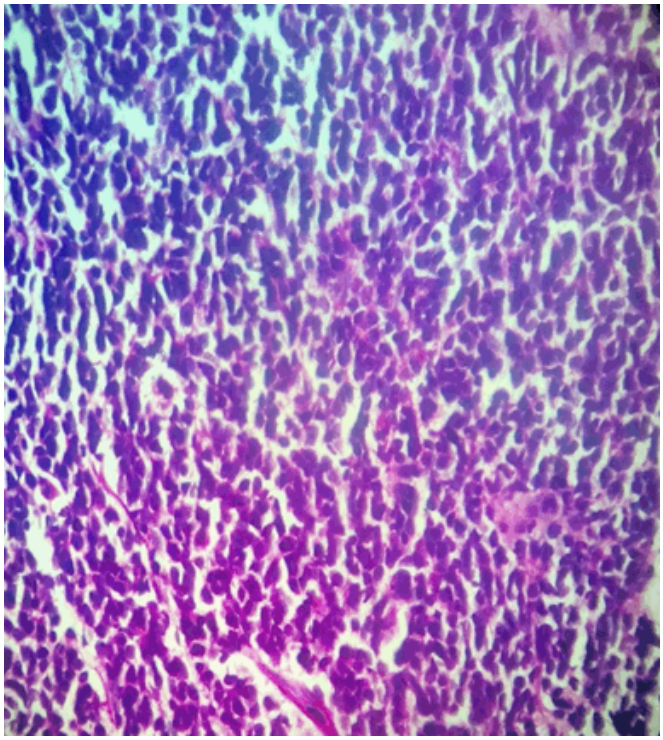

Figure 1: Classical Ewings sarcoma showing Lobular pattern of monomorphic round cells (H&E, X400)

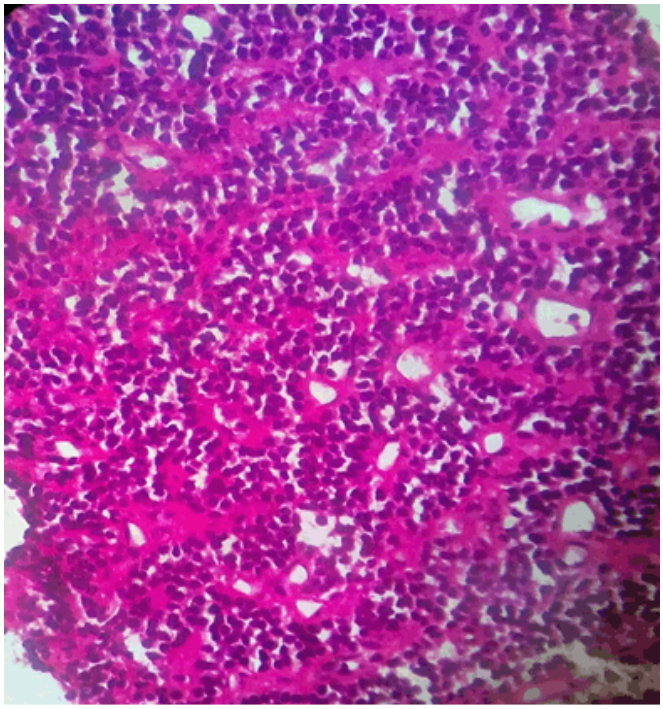

Figure 2: Primitive neuroectodermal tumor showing Homer-Wright rosettes (H&E, X400).

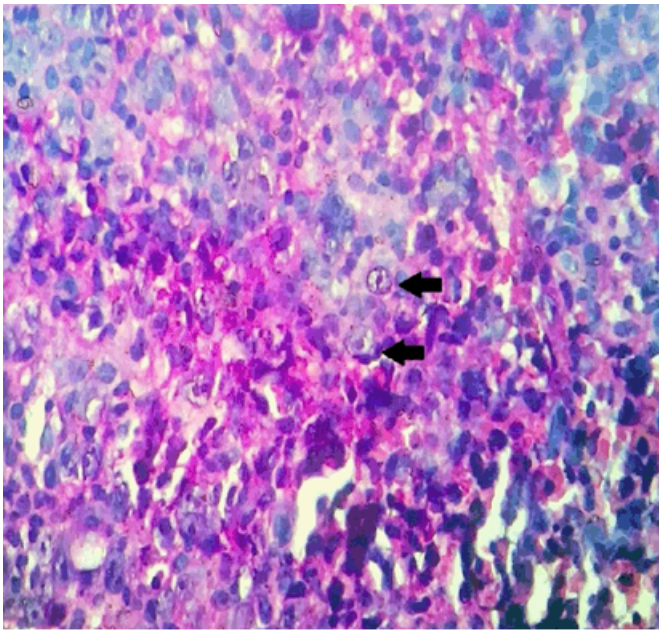

Figure 3: Atypical Ewings sarcoma showing large Cells with prominent nucleoli (H&E, 400).

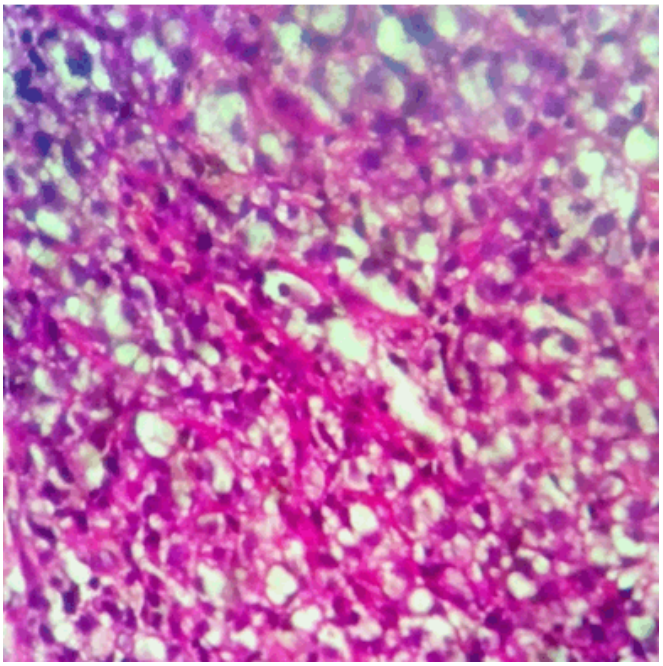

Figure 4: Atypical Ewings sarcoma showing tumor cells with abundant clear cytoplasm (H&E, 400).

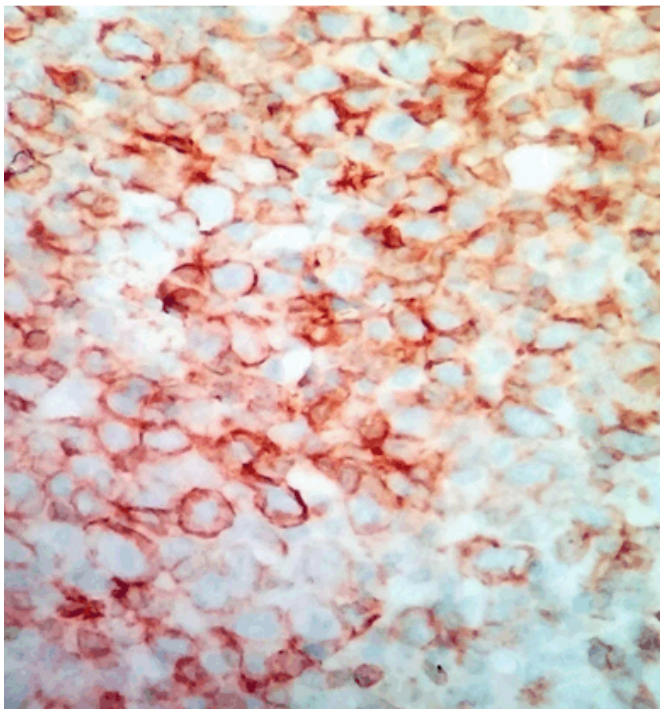

Figure-5: Strong membranous CD-99 positivity in Tumor cells (CD99, X400).

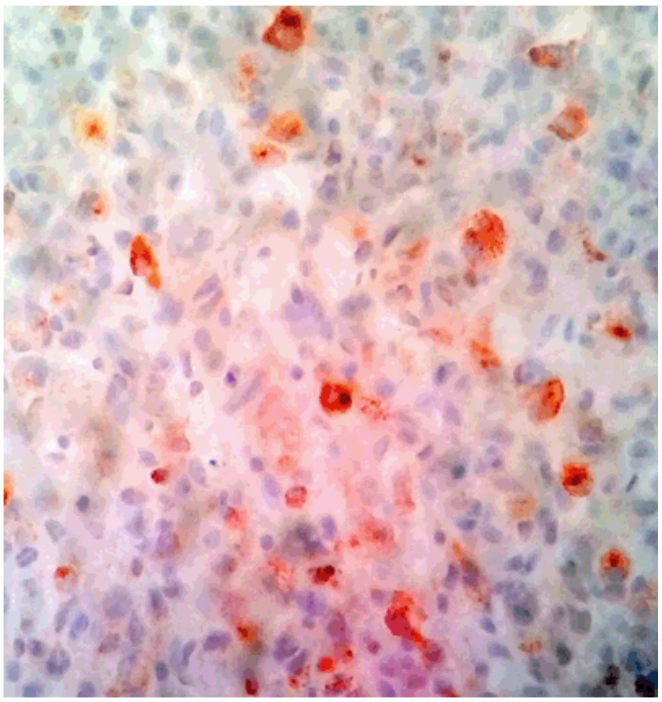

Figure-7: Atypical Ewings sarcoma showing Focal EMA positivity in clear cells (EMA, X400).

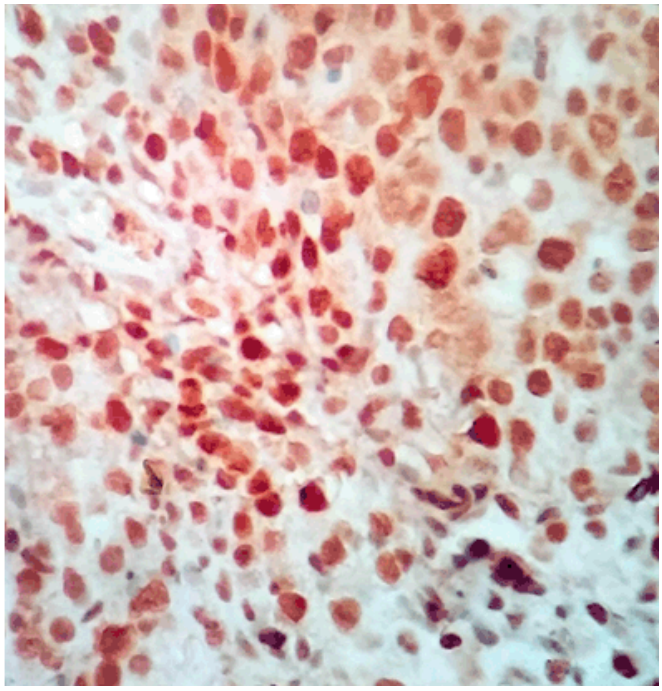

Figure-6: Tumor cell showing strong nuclear FLI-1 positivity (FLI-1, X400).

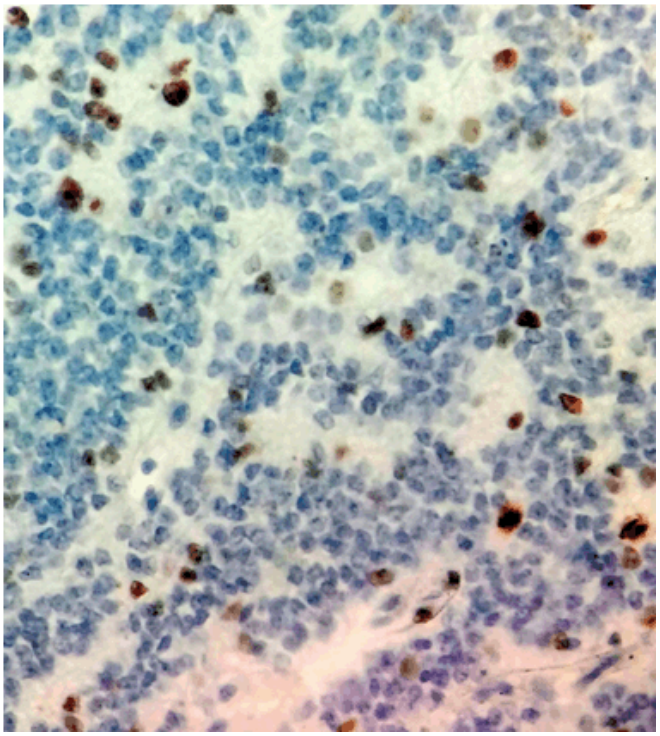

Figure-8: PNET showing nuclear MIB-1 positivity (MIB-1, X400)s.