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Role of Immunohistochemistry in Diagnosis of Undifferentiated Malignant Tumours

Authors
Dr Praveen Singh Dhakar*[1], Dr Jayanti Mehta[2], Dr Alka Mittal[3]
[1]MBBS, 3rd Year Resident, Department of Pathology, SMS Medical College, Jaipur
[2]MD, Professor, Department of Pathology, SMS Medical College, Jaipur
[3]MD, Senior Demonstrator, Department of Pathology, SMS Medical College, Jaipur
Corresponding Author
Dr Praveen Singh Dhakar
C-39, Resident Doctors Hostel, SMS Medical College, Jaipur- 302004 INDIA
Mob-9694443292, 7976569885, Email: *pearlstar30@gmail.com*

Abstract
Background: *Immunohistochemistry provides a true identitiy for undifferentiated tumours. The accurate typing of malignant and benign tumours are important for prognostic and therapeutic purposes.*
Aim: *The present study is designed*
1. *To identify the histogenesis of undifferentiated malignant tumours by immunohistochemical analysis.*
2. *To take out spectrum of tumours diagnosed as undifferentiated malignant, poorly differentiated and small round cell on histology.*

Materials & Methods: *A total of 92 cases diagnosed as undifferentiated malignant tumours, poorly differentiated malignant tumours as well as small round cell tumours on histopathology in the Department of Pathology, SMSMC Jaipur from October 2015 to January 2017 were taken and immunohistochemistry was applied to reach the final diagnosis.*
Results: *Its was possible to arrive at final diagnosis in 89 (96.73%) cases with the help of immunohistochemistry. Majority of the cases were of Mesenchymal lineage, among them Ewing's sarcoma/PNET was the most frequent diagnosis followed by Non Hodgkin's lymphoma (DLBCL) in hematopoietic lineage*
Conclusion: *Immunohistochemistry is valuable adjunct to H & E staining in diagnosis of undifferentiated tumours. Accurate typing is prognostically and therapeutically important, for that a panel approach of carefully selected antibodies is always recommended.*
Keywords: *Immunohistochemistry, Undifferentiated Malignant tumours, Small round cell tumours.*

Introduction

To classify the undifferentiated tumours, immunohistochemistry and electron microscopy are most important tools. With a basic panel of antibodies which are relatively specific lineage (epithelial, mesenchymal, lymphoid), we can usually classify a given neoplasm.[1] With the introduction of the immunohistochemical method, by Coons et al. in 1942[2],it has become a powerful complementary tool in tumour analysis. However, only since the early 1990s has the method found general application in surgical pathology[3-6]. Histologic subtyping with the help of immunohistochemical characterization of tumors

has resulted in a level of distinction between diagnoses which was not previously possible.[7] Through the identification of specific cellular components of cell patterns, using a specific panel of monoclonal or polyclonal antibodies, the immunohistochemical method has transformed the diagnosis of these tumors. For treatment purposes, it is important to know whether an undifferentiated tumor is epithelial, mesenchymal or hematopoietic.

Immunohistochemical dissection of undifferentiated tumours is also helped by categorizing them into small round blue cell tumors (SRCTs) or large cell tumours. The latter group is further divided into (1) carcinomatous tumours,(2) sarcomatous or sarcoma-like tumours, and (3) tumours with overlapping features[8-9].

In this prospective analysis, we evaluated the histogenesis of undifferentiated, poorly differnetiated malignant tumours and small round cell tumours and the way in which they were distributed, according to cell pattern, patient's age and tumour localization.

The role of immunohistochemical techniques in determining the conclusive diagnosis was also evaluated.

Material & Methods

The present study was undertaken in the department of pathology in SMS Medical College, Jaipur. Total 92 cases were taken for study from October 2015 to January 2017, which were reported as undifferentiated or poorly differentiated malignant tumours and small round cell tumours using routine Hematoxylin- eosin stain. There were 55 males and 37 females, their age ranged from 2 years to 78 years. The clinical history including age, gender and location of tumour were obtained from histopathology requisition forms. Representative sections of resected specimen were fixed in 10% buffered formalin and processed. After routine processing 4 um thick sections were cut and stained with hematoxylin- eosin method. For immunohisto-chemistry, the technique we used, was based on Peroxidase Antiperoxidase method. Sections were mounted on poly-l-lysine coated slides and deparaffinised with xylene and blocked for peroxidase with 3% H_2O_2. Antigen retrieval was done by De-clocking chamber using citrate buffer. A primary panel of antibodies consisting Cytokeratin (ck), Leucocyte common antigen (LCA), Vimentin, S100 was applied and incubated followed by secondary antibody and peroxidase antiperoxidase complex. 3-3' diaminobezidine tetrahydochloride (DAB) was used as a chromogen then counterstained by hematoxylin and mounted in distrene dibutylphthalate (DPX)[10-11].

Secondary panel of antibodies was used to reach the final diagnosis. Antibodies used for mesenchymal lineage are CD99, FLI-1, Myogenin, MyoD1, smooth muscle actin (SMA). For hematopoietic lineage CD10, CD15, CD20, CD30, Bcl2, Bcl6, Mum-1 were applied. For epithelial lineage cytokeratin (ck), carcinoem-bryonic antigen (CEA), epithelial membrane antigen (EMA), chromogranin (CGA) and for germ cell tumours, placental alkaline phosphatase (PLAP), alfa fetoprotein (AFP), CD117, CD30, OCT3/4 were applied. In the present study we considered weak/moderate/strong staining as positive staining.

Observations and Results

Among 92 cases, bone and soft tissue was most common site (46.74%) followed by gastroint-estinal tract (17.39%), respiratory tract (10.87%) and lymphoid tissue (09.78%).[Table 1]

Bone and soft tissue were the most common site for mesenchymal neoplasms. For hematopoietic neoplasms, bone and soft tissue and gastrointestinal tract were the most common site.

Respiratory tract and gastrointestinal tract were most common site for epithelial neoplasms.

Most frequent diagnosis was Mesenchymal (36.96%) followed by Epithelial (31.52%), Hematopoietic (28.26%) and Germ cell tumours (03.26%).[Table 2]

Among mesenchymal lineage most common affected age group was < 20years (52.94%). Hematopoetic lineage affected a wide age range group 21-60 years (69.24%). 41-60 years age group was commonly affected by epithelial lineage (48.27%). Germ cell tumours commonly seen in 21-40 years of age group (66.67%). [Table 3]

Among 34 cases of mesenchymal lineage majority of the cases were of younger age group <20 years (52.9%) followed by 11 cases (32.35%) in the age group 21-40 years. Among 26 cases of hematopoietic lineage majority of the cases were of diffuse large B- cell lymphoma falling in the wide age range group of 21-60 years (53.84%). Majority of the epithelial neoplasms were falling in the age group of 41-60 years (48.27%) followed by 7 cases in the age group of >60 years (24.13%).Only 3 cases were of germ cell tumours out of total 92 cases. Two were in 21-40 years of age group and one case was of >60 years of age.

Incidences were more in males than in females in all the lineages.

In present study out of 92 cases, 34 cases were of mesenchymal lineage. Among 34 mesenchymal cases, 21 cases (61.76%) were diagnosed as Ewing's sarcoma/PNET. All were positive for FLI-1, 20 out of 21 cases (95.23%) showed positivity for CD99, and 3 cases (14.28%) were positive for Ck.

Two cases were diagnosed as neuroblastoma, all were positive for NSE, one case was of esthesioneuroblastoma showed positivity for S-100 and one case was positive for CD56 and synaptophysin.

Two cases were of rhabdomyosarcoma, both were positive for Desmin, MyoD1, Vimentin, Myog-enin. One showed focal positivity for PanCk.

Two cases were diagnosed as synovial sarcoma, both were positive for Ck7, Bcl2 and vimentin.

One case of clear cell sarcoma (Vimentin+,S-100+), one case of epithelial sarcoma (CD34+, EMA+), one case of Gastrointestinal stromal tumour (CD117+), one case of Leiomyosarcoma (SMA+, H-Caldesmin+) and one case of Luteinized granulosa cell tumour (PanCk+, Calretinin+) were also diagnosed.

In one case we could not reach to the final diagnosis. That was diagnosed as malignant mesenchymal neoplasm, showed positivity for Vimentin only. We could not apply further panel of markers due to scant biopsy tissue. [Table 4]

Among 26 cases of hematopoietic lineage, 16 cases (61.53%) were diagnosed as Non Hodgkin's Lymphoma (DLBCL), all were positive for LCA and CD20. Out of 16, 11 cases (68.75%) were positive for Bcl6 and 8 cases (50%) were positive for Bcl2. One case of DLBCL originating from anal region was negative for Bcl2 and one case originating from stomach was negative for Bcl6. All cases of DLBCL showed high MIB index ranging from 60-90%.

Two cases (7.69%) of Burkitt's lymphoma were diagnosed, both were positive for LCA, CD20, CD79a. one case was positive for CD99 and Bcl2 and other one was negative.

Two cases (7.69%) were of classical Hodgkin's Lymphoma, both were positive for CD15,CD30 and MUM-1 and negative for LCA.

Two cases (7.69%) of Lymphoblastic lymphoma were diagnosed, both were positive for Tdt, CD20 and negative for LCA.

One case of primary anaplastic large T cell lymphoma (CD3+,MUM-1+, CD30+, Vimentin+) with MIB index 75% was diagnosed.

One case was of plasmblastic neoplasm that was positive for vimentin, MUM-1, and focally positive for CD30. Negative for CD3, CD20, CD38, Bcl2, CK, melan-A, CD79a, S-100. MIB score was 99%.

We could not further categorize one case of Lymphoma due to small biopsy. That was LCA positive. [Table 5]

Among 29 cases of epithelial lineage, 5 cases (17.24%) were of adenocarcinoma. Out of 5, 3 cases (60%) were positive for ck, all were positive for Ck7 and one case was of metastatic adenocarcinoma from lung, that was positive for NapsinA and TTF.

4 cases (13.79%) were of malignant melanoma. All four cases were positive for Melan A and S-100. Two cases were positive for HMB45 and vimentin, and rest two were negative.

4 cases (13.79%) were of nasopharyngeal carcinoma. All were positive for ck and 3 cases were positive for P63.

One case was of renal cell carcinoma that was positive for Panck, vimentin, CD10.

4 cases (13.79%) were diagnosed as squamous cell carcinoma. All were positive for ck and P63

Three cases (10.34%) were diagnosed as undifferentiated carcinoma. All were positive for ck and two were positive for EMA.

One case was diagnosed as metastatic carcinoma on liver biopsy that was positive for ck. We could not apply further panel of markers due to scant biopsy tissue. [Table 6]

Out of 92 cases, 3 cases (3.26%) were of germ cell tumours.

One case of spermatic seminoma was positive for NSE only. It was negative for PLAP, CD117, CD30, OCT3/4, CD20, CD3.

One case of metastatic germ cell tumour was diffusely positive for Panck and focally positive for AFP. It was negative for TTF, CD117, CD30 and OCT ¾.

One case of mixed germ cell tumour was positive for PLAP, CD117, and AFP. It was negative for CD30. [Table 7]

Table 1: Distribution of cases on the basis of Location of tumour

	No. of cases	Percentage
Bone and soft tissue	43	46.74%
Gastrointestinal Tract	16	17.39%
Lymphoid tissue	9	9.78%
Respiratory tract	10	10.87%
Male genital tract	1	1.09%
Female genital tract	6	6.52%
Skin	1	1.09%
Central nervous system	2	2.17%
Kidney	1	1.09%
Adrenal	1	1.09%
Lung	2	2.17%
Total	92	100%

Table 2: Distribution of cases according to lineage

	No. of cases	Percentage
Mesenchymal	34	36.96%
Hematopoietic	26	28.26%
Epithelial	29	31.52%
Germ cell	3	3.26%
Total	92	100.00%

Table 3: Distribution of cases of various age groups according to lineage

	Mesenchymal	%	Hematopoietic	%	Epithelial	%	Germ cell	%
<20	18	52.94	4	15.38	2	06.90	-	-
21-40	11	32.36	9	34.62	6	20.69	2	66.67
41-60	2	5.88	9	34.62	14	48.27	-	-
>60	3	8.82	4	15.38	7	24.14	1	33.33
Total	34	100.00	26	100.00	29	100.00	3	100.00

Table 4: Categorization of Mesenchymal Neoplasms on Primary panel

Mesenchymal neoplasms		**Primary panel**			
	No.	**CK**	**LCA**	**Vimentin**	**S-100**
Clear Cell Sarcoma	1	-	-	1/1	1/1
Epithelial sarcoma	1	1/1	-	1/1	-
Ewing's Sarcoma/PNET	21	3/21	-	20/21	-
Gastrointestinal stromal tumour	1	-	-	1/1	-
Leiomyosarcoma	1	-	-	1/1	-
Lutenized Granulosa cell tumour	1	-	-	1/1	-
Malignant mesenchymal neoplasm	1	-	-	1/1	-
Neuroblastoma	2	-	-	-	-
Esthesioneuroblastoma	1	-	-	-	1/1
Rhabdomyosarcoma	2	-	-	1/2	-
Synovial sarcoma	2	2/2	-	2/2	-
Total	34	6/34	-	29/34	2/34

Table 5: Categorization of Hematopoietic Neoplasms on Primary panel

Hematopoietic neoplasms		**Primary panel**			
	No.	**CK**	**LCA**	**Vimentin**	**S-100**
Burkitt's lymphoma	2	-	2/2	-	-
Classical Hodgkin's Lymphoma	2	-	-	-	-
Lymphoblastic lymphoma	2	-	-	1/2	-
Lymphoma	1	-	1/1	-	-
Non Hodgkin's B cell Lymphoma/small cell type	1	-	1/1	-	-
Non Hodgkin's Lymphoma(DLBCL)	16	-	16/16	1/16	-
Plasmablastic neoplasm	1	-	-	1/1	-
Primary cutaneous anaplastic large T-cell lymphoma	1	-	1/1	1/1	-
Total	26	-	21/26	4/26	-

Table 6: Categorization of Epithelial Neoplasms on Primary panel of antibodies

Epithelial neoplasms		**Primary panel**			
	No.	**CK**	**LCA**	**Vimentin**	**S-100**
Adenocarcinoma	5	3/5	-	-	-
Malignant melanoma	4	-	-	2/4	4/4
Metastatic carcinoma	1	1/1	-	-	-
Nasopharyngeal carcinoma	4	4/4	-	1/4	-
Neuroendocrine carcinoma	6	4/6	-	-	-
Renal cell carcinoma	1	1/1	-	1/1	-
Squamous cell carcinoma	5	5/5	-	1/5	-
Undifferentiated carcinoma	3	3/3	-	-	-
Total	29	21/29	-	5/29	4/29

Table 7: Categorization of Germ cell tumours on Primary panel of antibodies

Germ cell tumours		Primary panel			
	No.	CK	LCA	Vimentin	S-100
Spermatic seminoma	1	-	-	-	-
Metastatic Germ cell tumour	1	1/1	-	-	-
Mixed Germ cell tumour	1	-	-	-	-
Total	3	1/3	-	-	-

Table 8: Comparison of location wise distribution of cases with other studies

Site	Pity Is et al[15]	Vasudha bhagat et al[16]	Present study
Bone and soft tissue	14.90%	24.32%	46.74%
Gastrointestinal Tract	19.70%	20.37%	17.39%
Lymphoid tissue	14.90%	13.51%	9.78%
Respiratory tract	23.60%	13.51%	10.87%
Male genital tract	-	9.48%	1.09%
Female genital tract	-	8.11%	6.52%
Skin	-	2.70%	1.09%
Central nervous system	-	1.35%	2.17%
Kidney	-	1.35%	1.09%
Adrenal	-	1.35%	1.09%
Lung	-	1.35%	2.17%

Table 9: Comparison of distribution of cases with other studies

	Non Hodgkin's Lymphoma	Carcinoma	Sarcoma	Neuroblastoma	Germ cell tumour
Coindre et al[12]	57%	22%	5%	-	-
Bashyal R et al[14]	52.50%	5%	35%	2.50%	-
Pity Is et al[15]	17%	27%	13%	-	-
Vasudha et al[16]	24.32%	36.50%	18.92%	4.05%	1.35%
Present study	21.74%	27.17%	30.43%	2.17%	3.26%

Table 10: Comparison of conclusiveness of immunohistochemistry with other studies

Coindre et al[12]	Durga s vege et al[13]	Pity Is et al[15]	Vasudha et al[16]	Present study
90%	85.50%	88.20%	98.65%	96.73%

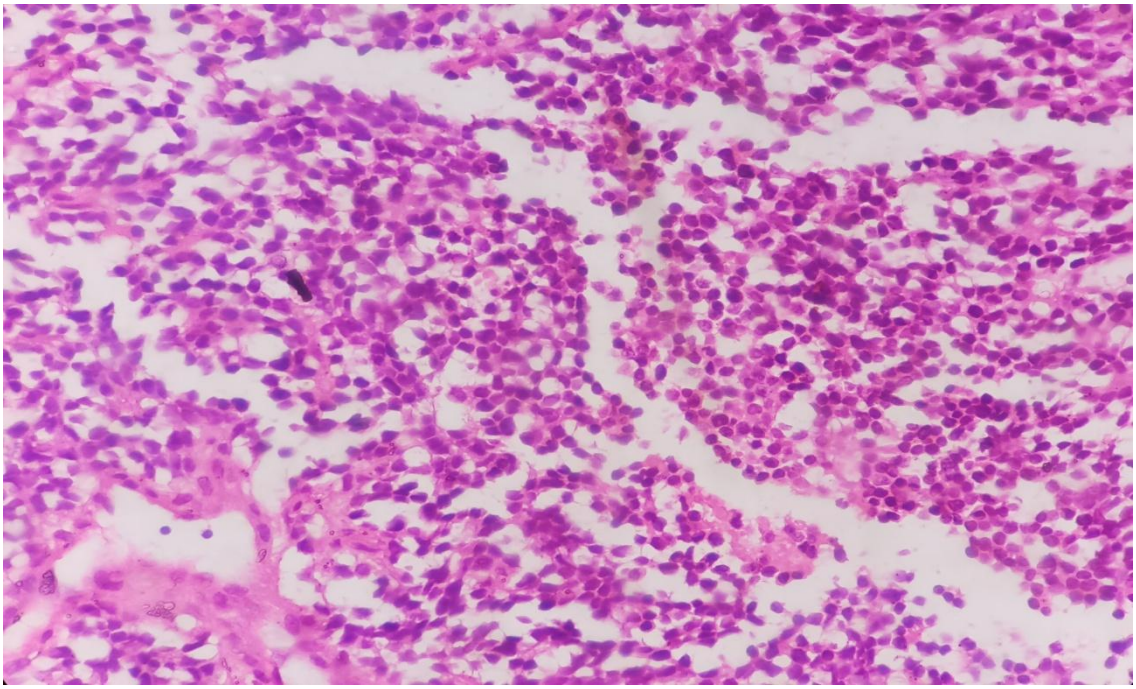

Figure 1- Ewing's sarcoma. Sheets of small, round, uniform cells with scant cytoplasm [H&E 400x]

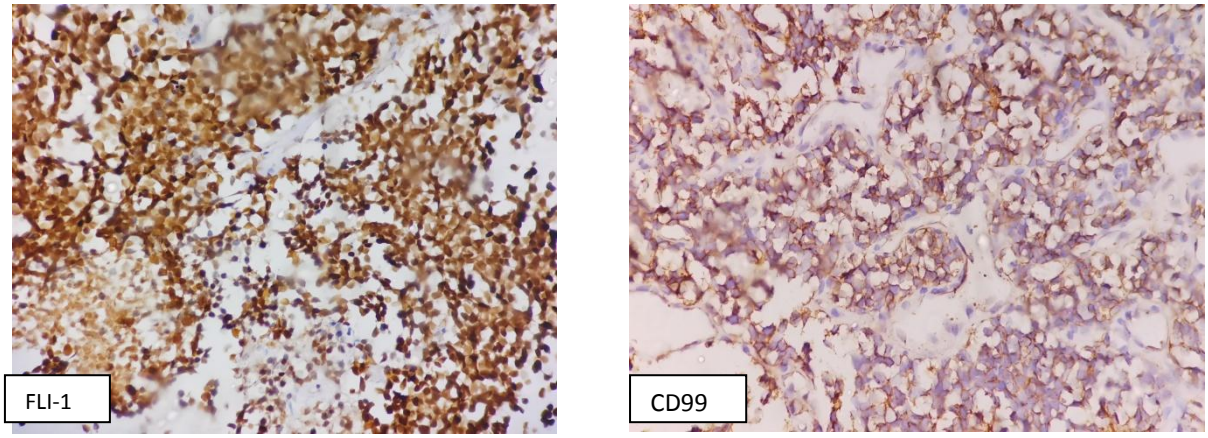

Figure 2- Ewing's sarcoma. FLI-1 – nuclear positivity, CD99 - membranous positivity

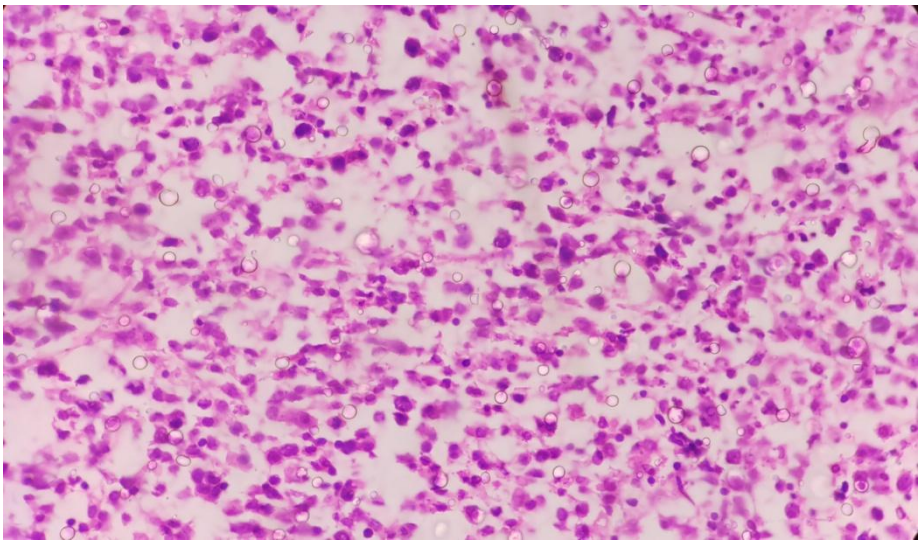

Figure 3- Diffuse Large B-cell Lymphoma. Diffuse growth of large, hyperchromatic and pleomorphic cells [H&E 400X]

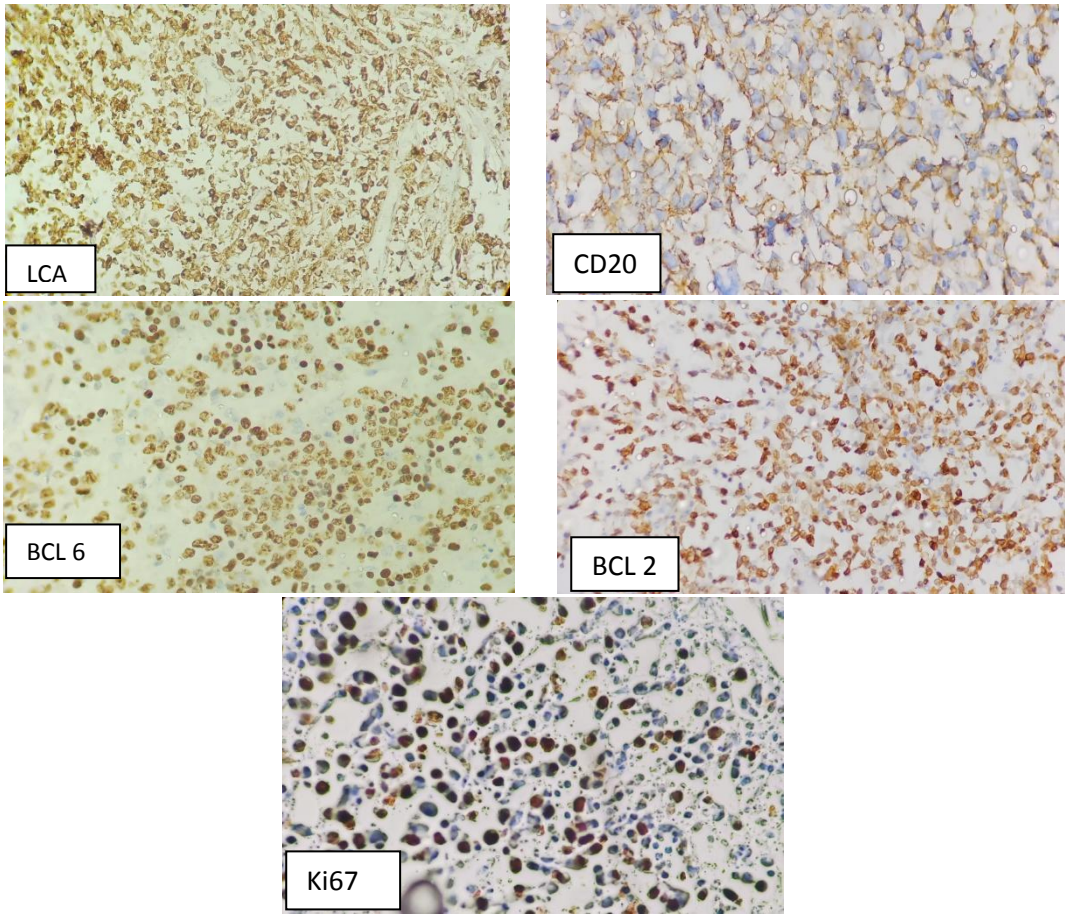

Figure 4- Diffuse Large B cell Lymphoma. LCA- cytoplasmic positivity. CD20- membranous positivity, Bcl6- nuclear positivity and Bcl2 shows nuclear as well as cytoplasmic positivity

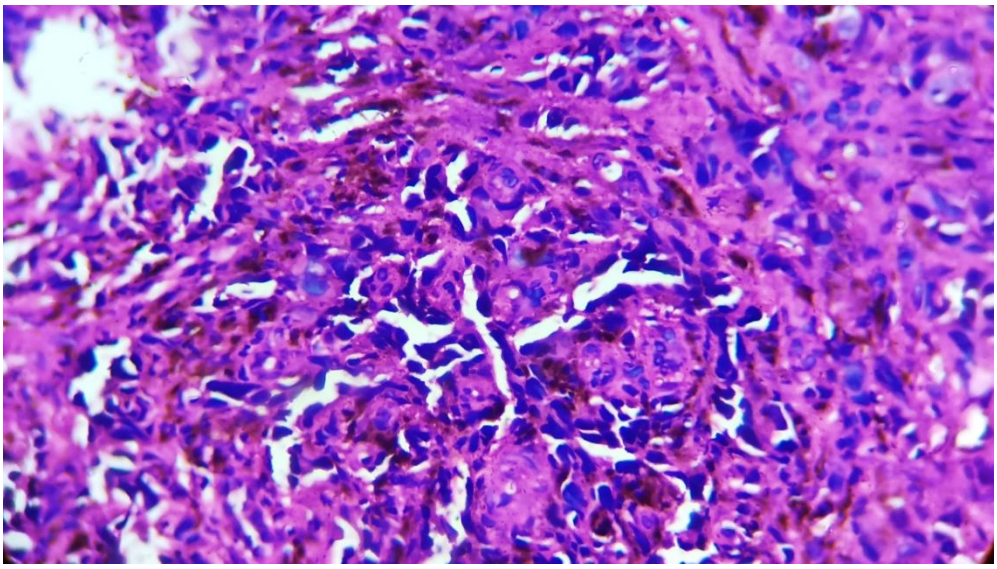

Figure 5- Malignant melanoma. Malignant cells having large pleomorphic, hyperchromatic nuclei with eosinophilic cytoplasm. Melanin pigment is present. [H&E 400X]

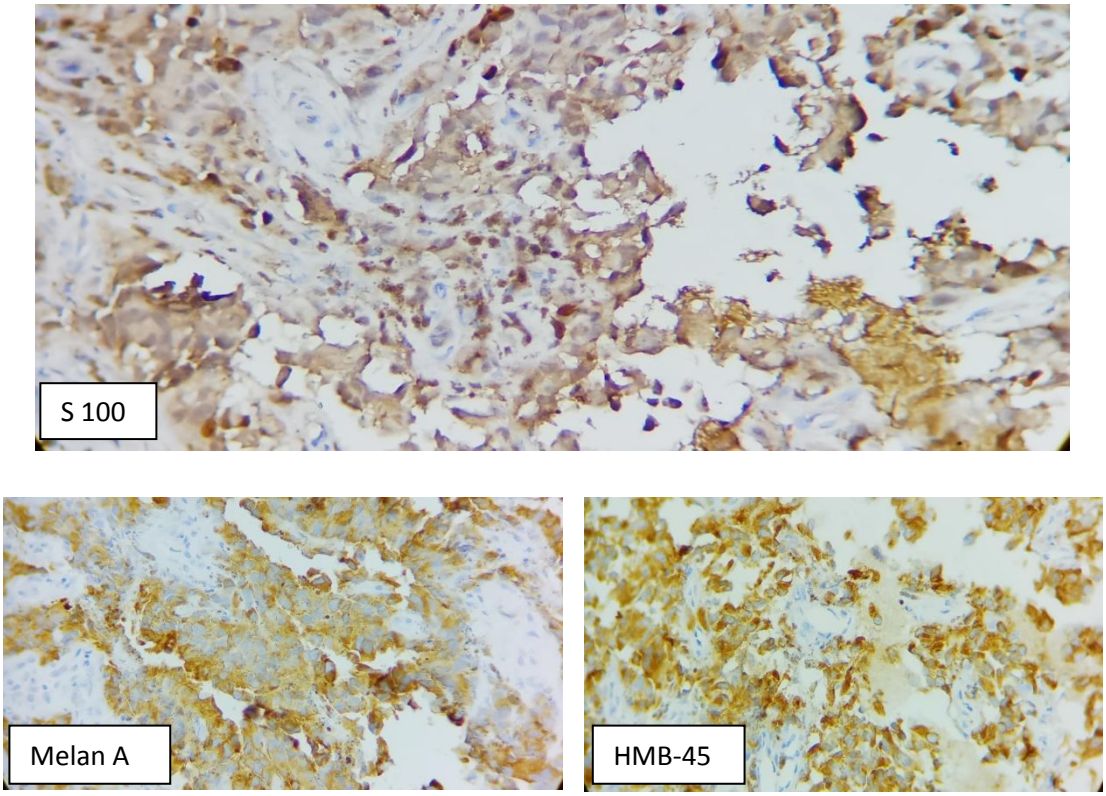

Figure 6- Malignant melanoma. S-100 - both nuclear and cytoplasmic positivity. Melan A and HMB-45 - cytoplasmic positivity

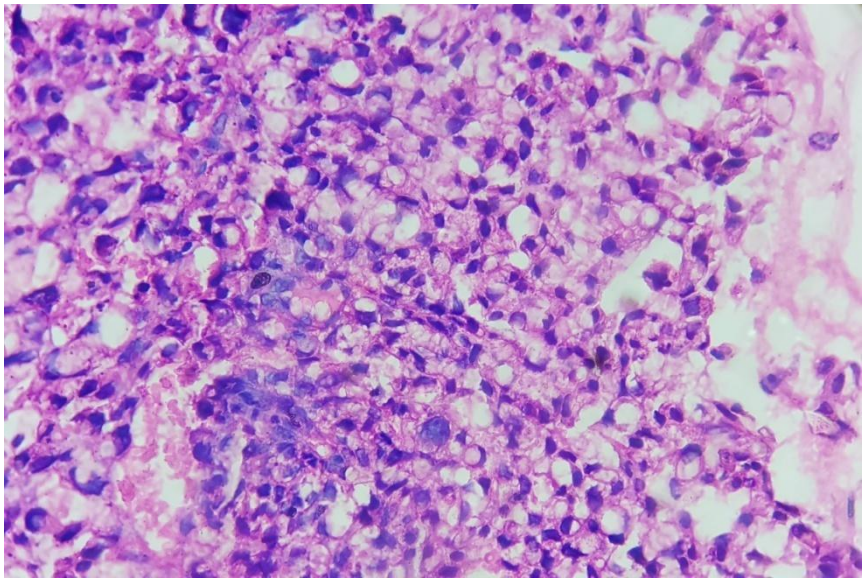

Figure 7- Mixed germ cell tumour. Two types of pattern of cells, Seminomatous and microcystic pattern of yolk sac tumour is present

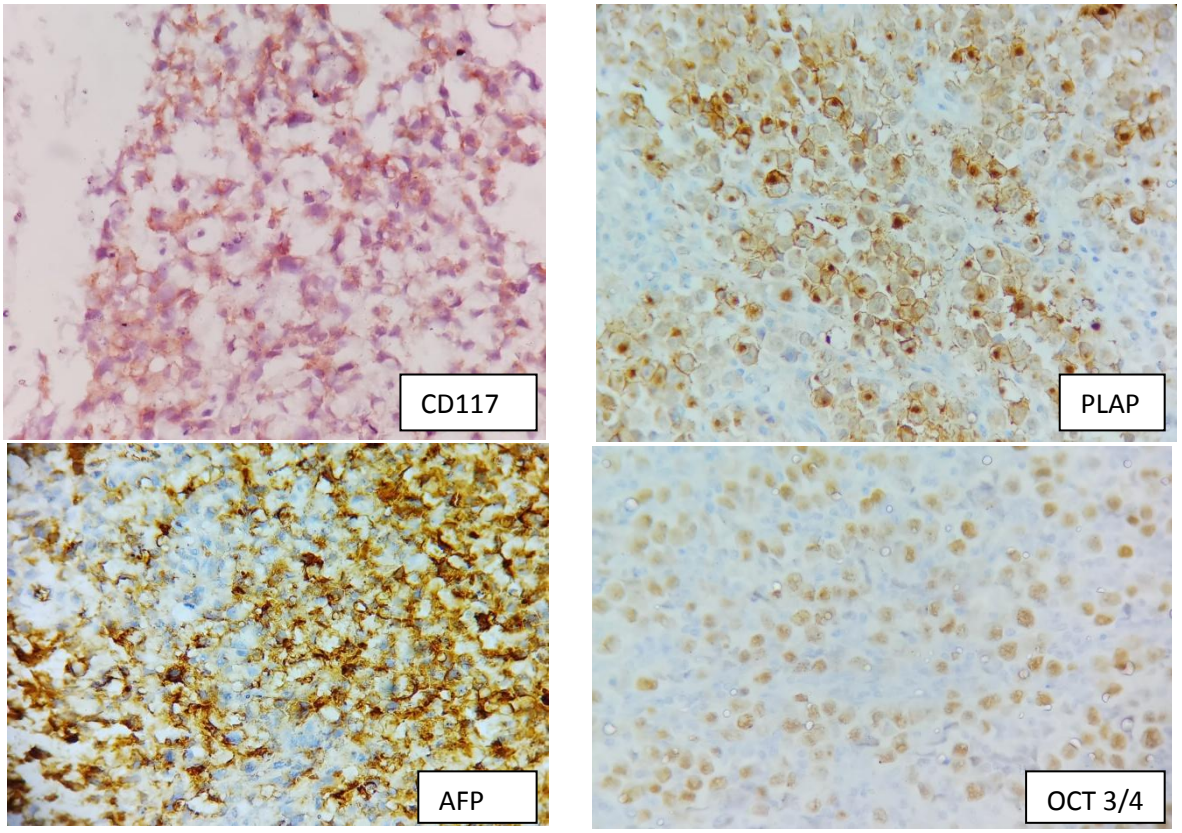

Figure 8- Mixed germ cell tumour shows seminomatous and yolk sac component. CD117 and PLAP - membranous positivity. AFP – cytoplasmic positivity and OCT3/4 shows nuclear positivity

Discussion

In our study of 92 cases of undifferentiated malingnant tumours, most common site was bone and soft tissue (46.74%) followed by gastrointestinal tract (17.39%) and lymphoid tissue (9.78%). This finding was consistent with the finding of vasudha bhagat et al[16]. Pity Is et al[15] had the different findings as they have included only small round cell neoplasms in their study.[Table 8]

In our study, cases of Non Hodgkin's Lymphoma were 21.74%. This finding is consistent with the study of Vasudha et al[16] and Pity Is et al[15]. However Coindre et al[12] and Bashyal R et al[14] had different findings which may be due to different sample size.

Cases of carcinoma were 27.17%. This finding is in accordance to the study of Coindre et al[12], Pity Is et al[15] and Vasudha et al[16].

Sarcoma cases were 30.43% in our study. This finding is consistent with the study of Bashyal R et al[14].

Total cases of Neuroblastoma in our study were 2.17%. Our finding is in accordance to the study of Bshayal R et al[14] and Vasudha et al[16].

Total cases of germ cell tumours in our study were 3.26%. This finding is consistent with the study of Vasudha bhagat et al[16].

In our study, overall incidences were observed more in males. This finding is consistent with Walter Adriano bianchini et al[17].[Table 9]

we could reach to the final diagnosis in 89 cases (96.73%) out of 92 cases in our study. In 3 cases we could diagnose the lineage only, due to scant biopsy tissue we could not apply further panel of markers. Our finding was in accordance to Vasudha Bhagat et al[16], Coindre et al[12], Pity Is et al[15] and Durga s vege et al[13][Table 10]

Summary and Conclusions

The present study was conducted in Department of pathology, SMS medical college, jaipur from October 2015 to January 2017. 92 cases of undifferentiated malignant tumours, poorly differentiated malignant tumours and small round

tumours were taken for the study purpose. Role of immunohistochemistry for exact histogenesis and spectrum of undifferentiated malignant tumours were studied.

- Among 92 cases most common site was bone and soft tissue followed by gastrointestinal tract, respiratory tract and lymphoid tissue respectively.
- Incidences were more in men compared to women.
- Most common affected age group for mesenchymal lineage was < 20 years, for hematopoietic lineage it was 21-60 years , 41-60 years for epithelial and 21-40 years was for germ cell tumours.
- Majority of the cases were of mesenchymal lineage, among them Ewing's sarcoma/PNET was the most frequent diagnosis followed by Non Hodgkin's lymphoma (DLBCL) in hematopoietic lineage.
- We were able to reach for final diagnosis in 96.73% cases with the help of immunohistochemistry.

So here we reached on the conclusion that immunohistochemistry is valuable adjunct to H & E staining in diagnosis of undifferentiated tumours showing overlapped histologic features. The accurate typing of malignant and benign tumours are important for prognostic and therapeutic purposes. For accurate characterization of tumours, a panel approach of carefully selected antibodies is always recommended. In recent years better understanding of molecular studies of these tumours allow us to do molecular testing to provide rapid and comprehensive solutions for questionable cases.

Conflict of Interest- None

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