ORIGINAL RESEARCH PAPER
Volume - 12 | Issue - 06 | June - 2023 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AN OBSERVATIONAL STUDY OF THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE ANALYSIS OF MALIGNANT SMALL ROUND CELL TUMORS

Pathology

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ABSTRACT

Background: Small round cell tumors (SRCT) are heterogeneous malignancies featuring primitive undifferentiated small cell morphology and describe the group of highly aggressive malignant tumors composed of relatively small and monotonous undifferentiated cells with increased nuclear-cytoplasmic ratio. Immunohistochemistry (IHC) has emerged as the most practical adjunct tool to histopathology making accurate diagnosis possible. **Aim:** To study the role of Immunohistochemistry in the analysis of malignant small round-cell tumors. **Objectives:-**(1) To study the importance of immunohistochemistry profile in malignant small round cell tumors. (2) Categorize the malignant small round cell tumors by Immunohistochemistry. (3)To know the age and sex incidence of malignant small round cell tumors. (4) To correlate histopathological and IHC features. **Materials and Methods:** Total 90 cases had been reported as Malignant Round Cell Tumor on biopsy.during the study period 2021-22. Biopsy tissues/ samples were fixed, paraffin embedded, sectioned and, stained with hematoxylin and eosin. IHC was performed on each case. Results were analyzed and compared. **Results:** Out of 90 cases, There were 31 cases(34.4%) of Small Cell Carcinoma with the highest incidence, followed by 28 cases (31.1%) of Non-Hodgkin's lymphoma, Synovial Sarcoma 10 cases (11.1%), Ewings Sarcoma 8 cases(8.9%),Neuroblastoma 5 cases(5.6%), Malignant Melanoma 4 cases (4.4%), Rhabdomyosarcoma 3 cases(3.3%) and 1 case of Wilms Tumor. **Conclusion :-** Malignant Small Round Cell Tumors comprise a wide range of Heterogeneous group of Tumors. On light microscopy, these tumors have the basic morphologic themes of small round blue cells. In the diagnosis of Malignant small Round Cell Tumors, , light microscopic findings is basic but not exact categorization done in many Undifferentiated Tumors. For diagnosis and exact categorization Immunohistochemistry are all taken into consideration. Immunohistochemical marker study has a great role of categorizing these tumors and accurate diagnosis is made possible which enable the patients to get the appropriate treatment and for better Prognosis

KEYWORDS

Immunohistochemistry Malignant Small RoundCellTumor ,Non Hodgkin's Lymphoma, Ewing's Sarcoma, Rhabdomyosarcoma

INTRODUCTION

Small round cell tumors (SRCT) are heterogeneous malignancies featuring primitive undifferentiated small cell morphology[1] and describe the group of highly aggressive malignant tumors composed of relatively small and monotonous undifferentiated cells with increased nuclear-cytoplasmic ratio.

The group includes primitive neuroectodermal tumor (PNET),Ewing's sarcoma family neuroblastoma, rhabdomyosarcoma, desmoplastic small round cell tumor, Non-Hodgkin's lymphoma, small cell osteosarcoma, small cell carcinoma (undifferentiated or neuro-endocrine), medulloblastoma, synovial sarcoma, mesenchymal-chondrosarcoma, Merkel cell carcinoma, Wilm's tumor, and retinoblastoma[2].

Other differential diagnoses of small round cell tumors include undifferentiated hepatoblastoma, granulocytic sarcoma, and Intra abdominaldesmoplastic small round cell tumor[3].

Typically, a multimodal approach is employed and the principal ancillary techniques that are useful in classification are immuno-histochemistry and immunophenotyping by flow cytometry, reverse transcriptase polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and electron microscopy. [1]

Immunohistochemistry (IHC) has emerged as the most practical adjunct tool to histopathology making accurate diagnosis possible[4]. The occurrence of these tumors and how they were distributed according to the localization were also evaluated. IHC markers – CD 99, NSE, Vimentin, S-100, Desmin, EMA, LCA, CD 10, CD 20, CD 3, CD 5, etc applied. IHC represents a tool that can provide a clear distinction among the various tumor types.

The purpose of the study to categorize the patients of malignant small round cell tumors to ensure an appropriate and specific treatment as well as to identify the tumors which are at higher risk of recurrence and fatal outcome.

AIMS

To study the role of Immunohistochemistry in the analysis of malignant small round-cell tumors.

Objectives

1. To study the importance of immunohistochemistry profile in malignant small round cell tumors.
2. Categorize the malignant small round cell tumors by Immunohistochemistry.
3. To know the age and sex incidence of malignant small round cell tumors.
4. To correlate histopathological and IHC features.

MATERIALAND METHODS

Total 90 cases had been reported as Malignant Round Cell Tumor on biopsy.During the study period 2021-22. Biopsy tissues/ samples were fixed, paraffin embedded, sectioned and, stained with hematoxylin and eosin. IHC was performed on each case. Immunohistochemistry applied on biopsy by per oxidase method. Many markers according to provisional histomorphological diagnosis was applied. The Markers were CD 99, NSE, Vimentin, S-100, Desmin, EMA, LCA, CD 10, CD 20, CD 3, CD 5 applied. Results were analyzed and compared.

RESULTS AND OBSERVATION

The Malignant small round cell tumors are a heterogeneous group of malignant neoplasms Out of 90 cases, There were 31 cases(34.4%) of Small Cell Carcinoma with the highest incidence, followed by 28 cases

(31.1%) of Non-Hodgkin's lymphoma, Synovial Sarcoma 10 cases (11.1%), Ewings Sarcoma 8 cases(8.9%),Neuroblastoma 5 cases(5.6%), Malignant Melanoma 4 cases (4.4%), Rhabdomyosarcoma 3 cases(3.3%) and 1 case of Wilms Tumor.

Out of the 90 cases of Malignant Round Cell Tumors, 65.56% (59 cases) occurred in males and 34.44% (31 cases) in females with the male to female ratio of (1.9:1).

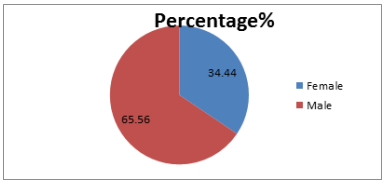
According to age wise distribution, the highest incidence was observed in 40-60 years of age group. According to sex wise distribution, a higher incidence was observed in males. Out of the 90 cases of Malignant Round Cell Tumors, 65.56% (59 cases)occurred in males and 34.44% (31 cases) in females with the male to female ratio of (1.9:1).. IHC study of Small Cell Carcinoma showed CD 56 (94.74%) Positivity, Synaptophysin showed 100% positivity NHL cases showed CD20 was Exclusively 100% (28/28 cases) positive , followed by Bcl-6 was 83.3% (20/24 cases) along with Bcl-2. Synovial sarcoma cases were showed different positive reactivity, TLE-1 was Exclusively 100% (10/10 cases) positive, followed by EMA with 80% reactivity. Ewing sarcoma cases were showed .CD99 was positive in 5 cases showed 71.4% reactivity. Two cases confirmed by NKX2.2 marker positivity and one was Synaptophysin positive. Neuroblastoma cases, Synaptophysin was 100% (5/5 cases) positive reactivity, along with CD56 in applied (2/2) cases. Malignant Melanoma cases showed, Melan-A 100% (4/4 cases) positive reactivity with HMB-45 (2/2) on Applied cases.and Rhabdomyosarcoma cases were showed 100% positive reactivity for Desmin, Vimentin, Myogenin.

One Case of wilms tumor is shows Pan- CK and WT-1 Positive .

Small cell carcinoma 16 cases (51.6 %) were found in 40-60 years and 14 cases (45.16%) >60 years of age. NHL 9 cases (32.14%) were found in 40-60 years and 11 cases(39.2%) in >60 years of age.

Synovial sarcoma and Ewing's sarcoma were found predominantly in11 -30 years of age. Neuroblastoma, Wilm'sTumor ,and Rhabdomyosarcoma were found in Paediatric age group in this study.

Distribution Of Cases According To Gender



Out of the 90 cases of Malignant Round Cell Tumors, 65.56% (59 cases)occurred in males and 34.44% (31 cases) in females with the male to female ratio of (1.9:1) as illustrated in the Chart

Table1

S.No.	Tumor Type	Positive Markers	Positive/Total	Percentage(%)
1	Small Cell Carcinoma	CD 56	18/19	94.74
		Synaptophysin	25/25	100.00
		TTF-1	24/28	85.71
2	NHL	CD 20	28/28	100
		Bcl-2	10/12	83.33
		Bcl-6	20/24	83.33
		MUM-1	9/12	75.00
		PAX-5	8/8	100
3	Synovial Sarcoma	TLE-1	10/10	100
		Vimentin	3/3	100
		EMA	4/5	80
4	Ewings Sarcoma	CD 99	5/7	71.43
		Vimentin	5/5	100
5	Malignannt Melanoma	Melan-A	4/4	100
		HMB-45	2/2	100
		S-100	3/4	75
6	Neuroblastoma	Synaptophysin	5/5	100
		CD 56	2/2	100
7	Rhabdomyosarcoma	Desmin	3/3	100
		Myogenin	2/2	100
		Vimentin	2/2	100
8	Wilms Tumor	WT-1	1/1	100

Table-2 Final Diagnosis After Immunohistochemistry (n=90)

Tumor type	No. of cases	Percentage (%)
Small Cell Carcinoma	31	34.4
Non Hodgkin's lymphoma	28	31.1
Synovial Sarcoma	10	11.1
Ewing's Sarcoma / PNET	8	8.9
Neuroblastoma	5	5.6
Malingnant Melanoma	4	4.4
Rhabdomyosarcoma	3	3.3
Wilm's tumor	1	1.1
Total	90	

DISSCUSSION

The family of the neoplasm which are generally known as Small Round Cell Tumors (SRCT) has grown in an increasingly complex fashion in recent years Increasing burden of malignant tumor, including SRBCT, has necessitated the need for accurate diagnosis to determine the magnitude of the problem. Immunohistochemistry is, therefore, very important routine accessory tool in playing an increasing role in modern surgical pathology, specially in making diagnosis of SRBCT[13].

Results of the present study were obtained, analyzed and compared with other studies.

In this present study, the age of the patients with Malignant Round Cell Tumors ranged between 5 Months to 85 years, 16 Cases in children with 17.78% of incidence and 74 cases in adults with 82.22% of incidence. The Mean Age of Cohort group was 45.75 Years.

Deepsikha et al [6]also found age range between 2 to 85 Years and mean age 40.8 Years.

Most of the RMS cases are below 21 years with the mean age at diagnosis of 9.8 Years. This study correlates well with the study conducted by Patel et al[1].

Neuroblastoma and Wilms Tumor found in Pediatric age group in this study. It Concordance with Patel et al [1],Deepsikha et al[6].

The sex distribution and male:female ratio in our study was in concordance with various studies conducted before by Deepsikha et al[6], Patel et al1, Meghavi R Joshi et al[5]and Zhuvithsii z et al [7]. The present study, as well as, most of the previous studies show males are more affected.

D'cruze L et al [2]with a male preponderance (74% were males and 26% were female patients

This study shows a male preponderance of Small Cell Carcinoma, Non Hodgkin's lymphoma Malignant Melanoma and Rhabdomyosarcoma with a male: female ratio of 6.7:1,2.1:1,3:1 and 2:1.

Meghaviet al[5]and Patel et al1 in NHL a male preponderance with a male: female ratio of 2.2:1 and 3.4:1.

Small cell carcinoma showed similarly male prepordance in Yatabe et al[9], Ye B Cappel J et al[10], and Zheng G et al[11]studies.

Neuroblastoma showed female preponderance in current study. Meghaviet al[5]also showed female prepondarance.

In this study, most common site for round cell tumors was respiratory tract. Intisar S. Pity et al[18]observed that Malignant round cell tumors frequently found in respirstrytract.Gastrointestinal tract (Stomach & colon) is predominant extranodal site for NHL. Pity, S et al[18]and Ravi G Patel et al[1],Deepshikha et al[6] study was concordant to this study in the view of incidence of round cell tumors.Patel et a[1], Deepshikha et al[6] and Sajidhussain et al[8] studies were observed NHL incidence 29.3%, 26.6% and 26.1%, which was similar to this study showed 31.1%.

Ewings Sarcoma incidence were observed 8.3% and 8.7% in Deepshikha et al[6] and Sajidhussain et al[8] studies which was similar to present study(8.9%).

CD 56, Synaptophysin and TTF-1 IHC Markers showed positive reactivity in Small Cell Carcinoma cases. CD 56 and Synaptophysin were showed 94.74% and 100 % Immunoreactivity in Small Cell

Carcinoma cases in this study. Yatabe et al[9]and Zheng G et al[11]study observed 97% and 98.9% Immunoreactivity of CD 56 Marker in Small Cell Carcinoma cases.

Total 28 cases of NHL (DLBCL-Type) were categorized after Immunohistochemistry in this study. The NHL cases were found positivity for different IHC Markers- LCA,CD 20, Bcl 2,Bcl 6, Pax-5 and MUM-1.

CD 20 showed 100% Immunoreactivity for NHL in this study, followed by Bcl-6 and Bcl-2 reactivity were 83.3%. Deepshikhaet al[6]study observed 100% Immunoreactivity for CD 20 and Bcl-6 which is similar to current study. Meghavi R Joshi et al[5]and Ravi G Patel et al[1]studies also observed 100% Immunoreactivity of CD 20 in NHL(DLBCL-Type) cases. Which is concordant to this study.

Total 10 cases were categorized by IHC in this study. The Synovial Sarcoma cases were found positive Immunoreactivity to different IHC Markers- TLE-1, EMA, Vimentin, Bcl-2, Pan-CK.

TLE-1 and EMA IHC Markers were observed 100% and 80% Immunoreactivity in current study. Rekhi et al[12]and Foo et al[14]were observed 95.2% and 92% Nuclear Immunoreactivity of TLE-1 Marker in Synovial Sarcoma cases, which is similar to this study.

Rekhi et al[12] and Bashyal et al[13]were observed 76.4% and 95% Immunoreactivity of EMA Marker in Synovial Sarcoma cases, which is similar to this study.

Total 8 cases of Ewing Sarcoma were categorized by Immunohistochemistry. Ewing Sarcoma cases showed different Immunoreactivity of IHC Markers- CD 99, Vimentin, NKX2.2, Synaptophysin. CD 99 IHC Marker showed 71.4% Membranous Immunoreactivity for Ewing Sarcoma cases in this study and Vimentin IHC Marker observed 100% Cytoplasmic Immunoreactivity on applied Ewings Sarcoma cases. Meghavi R Joshi et.al[5],Ravi G Patel[1]and Chang TK et al[15]studies were observed 94%, 86.7% and 70% CD 99 Membranous Immunoreactivity for Ewing Sarcoma respectively.

Total 5 cases of Neuroblastoma were categorized by Immunohistochemistry in this study.

Neuroblastoma cases were showed different Immunoreactivity for CD 56 and Synaptophysin IHC Markers in this study. CD 56 and Synaptophysin were showed 100% Cytoplasmic Immunoreactivity in Neuroblastoma cases. Meghavi R Joshi et.al[5]and Ravi G Patel et al[1]studies were observed 85% and 57% Synaptophysin Cytoplasmic Immunoreactivity respectively in Neuroblastoma cases.

Total 4 cases of Malignant Melanoma were diagnosed and categorized after Immunohistochemistry. IHC Markers Melan-A, HMB-45, S-100, Sox-10 were showed different positivity.

Melan-A and HMB-45 were observed 100% positive Immunoreactivity, followed by 75% positivity of S-100 for Malignant Melanoma in this study.

Bashyalet al[13]study observed positivity of HMB-45, S-100 and Melan-A.

Total 03 cases of Rhabdomyosarcoma were finally diagnosed and categorized after Immunohistochemistry. IHC Markers- Desmin, Vimentin, Myogenin were found different positivity in Rhabdomyosarcoma cases. Desmin and Vimentin showed 100% Cytoplasmic Immunoreactivity in this study.Meghavi R Joshi et.al[5], Ravi G Patel[1]and RossiaAntinio G etal[17]Studies were observed concordant to this study for Desmin and VimentinImmunoreactivity in Rhabdomyosarcoma.

1 case of Wilms Tumor found in this study, which was positive Immunoreactivity for WT-1 Marker and showed positive for Pan-CK
In Present study the incidence of Small Cell Carcinoma was Highest followed by NHL, Synovial Sarcoma and other MSRCT.

Deepshikhaet al[6]study was concordant to this study in the view of incidence of round cell tumors.

Patel et al[1], Deepshikha et al[6]and Sajidhussain et al[8]studies were observed NHL incidence 29.3%, 26.6% and 26.1%, which was similar to this study showed 31.1%.

Ewings Sarcoma incidence were observed 8.3% and 8.7% in Deepshikha et al[6]and Sajidhussain et al[8]studies which was similar to present study(8.9%).

Neuroblastoma cases incidence was found 5.6% in present study which was similar to Sajidhussainet al[8]and D'Cruze et al[2]studies showed 5.1% and 4.7%.

Table-3

Study group	Total cases in which IHC was applied	Diagnosis on IHC	Percentage of diagnosed cases (%)
D'Cruze et al2	40	40	100
Bhagat et al16	74	73	98.6
Deepshikha et al6	60	59	98.3
Present Study	90	90	100

Total 90 cases were categorized by IHC Markers in present study. The contributory diagnostic was 100% in my study.

Present study was concordant with D'Cruze et al[2]and Bhagatet[al 16]with diagnostic contribution was 100% and 98.6% respectively.

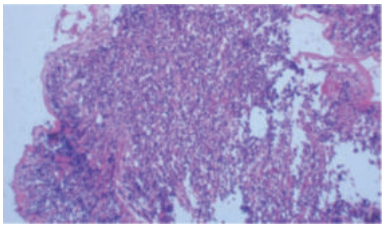


Figure 1 :- Small Cell Carcinoma (H&E Stain 100x)

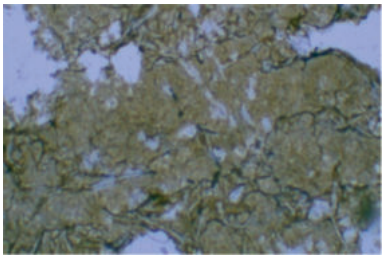


Figure 2 :- Synaptophysin Shows Cytoplasmic Immune Reactivity In Small Cell Carcinoma (100x)

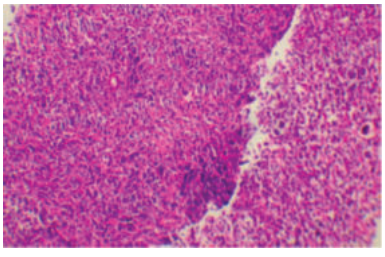


Figure 3 :- NHL (H&E Stain 100x)

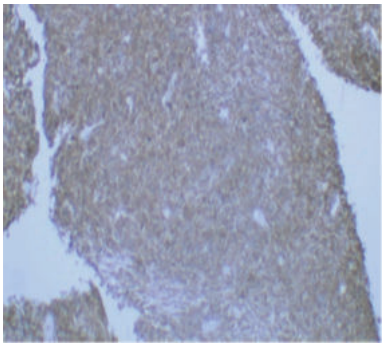


Figure 4 :- CD 20 Shows Cytoplasmic Immuno Reactivity In NHL (100x)

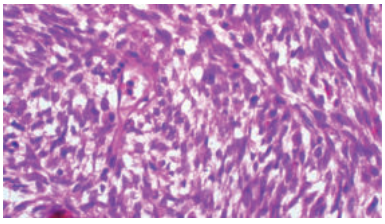


Figure 5 :- Synovial Sarcoma (h&e Stain 400x)

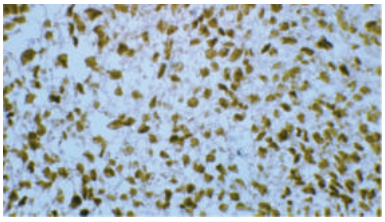


Figure 6 :- Tle-1, Shows Nuclear Immune Reactivity In Synovial Sarcoma (400x)

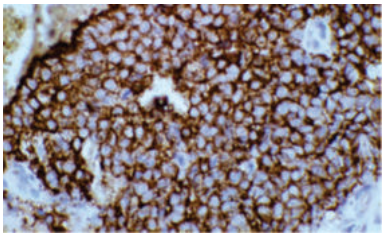


Figure 7 :- Cd 99 Shows Membranous Immune Reactivity Ewings Sarcoma (400x)

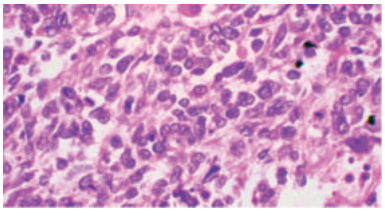


Figure 8 :- Rhabdomyosarcoma (h&e Stain 400x)

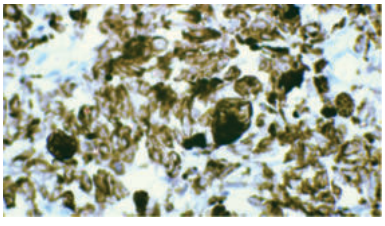


Figure 9 :- Desmin Shows Cytoplasmic Immune Reactivity In Rhabdomyosarcoma (400x)

CONCLUSION

Malignant Small Round Cell Tumors comprise a wide range of Heterogeneous group of Tumors. On light microscopy, these tumors have the basic morphologic themes of small round blue cells.

In the diagnosis of Malignant small Round Cell Tumors, , light microscopic findings is basic but not exact categorization done in many Undifferentiated Tumors. For diagnosis and exact categorization Immunohistochemistry are all taken into consideration.

Immunohistochemical marker study has a great role of categorizing these tumors and accurate diagnosis is made possible which enable the patients to get the appropriate treatment and for better Prognosis.

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