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ADJUNCTIVE ROLE OF IMMMUNOHISTOCHEMISTRY IN ANALYSIS OF SMALL ROUND CELL TUMOUR-A TERTIARY CARE CENTRE STUDY

Pathology

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ABSTRACT

"Small Round Cell Tumours" are a group of highly aggressive malignant neoplasms which feature small and monotonous undifferentiated cells with high nuclear cytoplasmic ratio on histology. As the treatment and prognosis vary greatly among these tumors accurate conclusive diagnosis is essential. A total of 76 cases were diagnosed as small round cell tumor on H&E stained section, which were further categorized using IHC profile. Final diagnosis of 88.16% cases of SRCT could be achieved on the basis of morphology and IHC evaluation, 75% cases required IHC for their subtyping while 25%could be accurately diagnosed on the basis of morphology and clinical corelation& 11.84% of cases remained unclassified. Conclusion: Immunohistochemistry is a very useful diagnostic tool in differential diagnosis of small round cell tumours in conjunction with morphological features and clinical parameters.

KEYWORDS

Small Round Cell Tumours, Immunohistochemistry

INTRODUCTION

The term **"Small Round Cell Tumours" (SRCT)** applies to a group of highly aggressive malignant neoplasms which feature the predominantly small and monotonous undifferentiated cells with high nuclear cytoplasmic ratio on histology. These are also called as **Small Blue Round Cell Tumours**, the name is derived from the primitive, highly cellular nature of these lesions, which typically present a vast sea of dark-blue nuclei in the sense that they have large hyperchromatic nuclei and a thin rim of cytoplasm on Haematoxylin & Eosin-based stains. Cytoplasmic abundance roughly correlates with cellular differentiation, which is often modest.[1,2]

Small round cell tumors may be associated with a variety of genetic factors, including constitutional mutations, deletions and epigenetic factors such as loss of imprinting, loss of heterozygosity, methylation abnormalities, histone acetylation errors, and dysregulation of non-coding RNAs. These lesions may also occur as acquired mutations, including "second hits", clonal progression, and translocations that produce chimeric proteins and altered promoter regions.

Their clinical presentations often overlap, therefore the differential diagnosis of Small Round Cell Tumors (SRCT) constitutes frequently a difficult diagnostic problem on routine H & E stained paraffin sections, especially if the primary origin is unknown or ill-defined.[3] It appears that biopsy specimens of various neoplasms may present morphology of Small Round Cell Tumors (SRCT).[4]

Considering the fact that both the treatment and the prognosis vary greatly among these tumors, a conclusive diagnosis is essential.[5] For treatment purposes and prognostic evaluation it is crucial to determine whether the malignant Small Round Cell Tumor (MSRCT) is epithelial, mesenchymal, neuroendocrine, melanocytic or hematopoietic in nature. But the anatomical locations and the microscopic details of these tumors and other aspects of their clinical presentations have a strong bearing on the relative likelihood of their respective diagnosis, and their immunohistochemical analysis may be tailored according to such considerations.

The histopathological assessment of Small Round Cell Tumors (SRCT) is the initial step of the diagnostic procedure because of appreciable similarity of SRCT morphological picture. In differential diagnosis the immunohistochemical studies are essential. In 80-90% of cases, diagnosis can be made on Haematoxylin & Eosin (H & E) staining slides coupled with Immunohistochemistry(IHC). However in 15- 20% there is a need for Molecular or Electron Microscopic diagnosis.

Immunohistochemistry is a key tool for the analysis of localization of target molecules within tissues. It has a significant role in the identification of tumors lacking evidence of lineage differentiation on the basis of routine light microscopic morphology alone. Approximately 90% of tumors posing diagnostic difficulties by morphology could be accurately classified by exploiting Immunohistochemistry. Several monoclonal antibodies have been developed, that detect different epitopes of the antigens which are present in the tumor cells. The use of extensive panels of antibodies in all malignant undifferentiated neoplasms allows accurate histological diagnosis in more than 89% cases.

However there is no antibody which is specific for a single tumor type and the pathologist has to judiciously correlate the clinical, radiological and the morphological findings with a panel of immumohistochemical (IHC) markers.

IHC represents a tool that can provide a clear distinction among the various tumor types. Its purpose is to categorize patients in order to ensure appropriate and specific treatment, as well as to identify tumors at higher risk of recurrence and fatal outcomes.

MATERIALS & METHODS

A total of 76 number of histopathologically diagnosed cases of Small Round Cell Tumours were studied prospectively from a period of January 2014 to June 2016 in the Department of Pathology, J.L.N. Medical College and Associated Group of Hospitals, Ajmer, Rajasthan. Formalin–fixed, paraffin-embedded sections of all the

cases which were diagnosed as small round –cell tumors on biopsies and resected specimens were studied. The patients belonging to all the age groups were included in the study. Decalcification was performed on the bony tissues before the routine processing. Haematoxylin and Eosin (H&E) stained sections of all the study cases were retrieved to confirm the tissue diagnosis, special stains was done wherever necessary and along with it the immunohistochemical stained sections were studied to categorize the tumors. The immunoprofiles selected for this study were Pancytokeratin(AE1/AE3),S100,CD45(LCA), Desmin,NSE,CD79a,WT1,PLAP,Vimentin.

Inclusion criteria- a)Cases diagnosed as Small Round Cell Tumors on light microscopy of all sites, in all age groups in both males and females were considered. b)Biopsies with adequate material to perform immunohistochemical analysis. c)Cases with all relevant clinical information regarding age, sex, site of tumour, radiological details etc. Exclusion criteria- Poorly preserved specimens were excluded.

Sample size- A total of 80 cases of small round cell tumours were received in our department during January 2014 to June 2016. 76 cases completely satisfied the inclusion criteria of our study, 4 cases were excluded as 1 case was received without fixative and was autolysed, 1 case showed inadequacy of tissue to perform IHC analysis and 2 cases had lack of relevant clinical details.

After fixation multiple bits were taken from representative areas of the tumor and the accompanying tissue .They were processed for histopathological examination and paraffin blocks were made. The blocks were then cut at 4-5 micron thickness and stained with Haematoxylin and Eosin. (H&E)

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The Poly-L-Lysine coated representative sections were labeled appropriately & sections were placed on hot plate at 70ºC for 15 minutes or 65 ºC for 20 minutes, till wax was dissolved and then successively passed through 2 changes of xylene solutions and ascending grades of alcohol solution. Sections were then washed in tap water for 1-2 min each & placed in PBS buffer. Peroxide Block: Endogenous peroxide quenching was performed by adding 3% H_2O_2 for 15-20 min. Later sections were washed in PBS buffer 3 changes, 2 min each. Antigen retrieval was done in pressure cooker{ pressure : 0.8 - 1 bar (11.6 - 15 psi), temperature : 121 °C (250°F)} / Temperature Controlled Antigen Retrieval System{ 1st cycle at 95^{0}C for 10min.; 2nd cycle at 98^{0}C for 05 min.}. Tanks containing slides were allowed to cool in Air conditioned till it reached room temperature and washed in PBS buffer 3 changes for 2 min each, then blot dry. Then tissues were encircled with Hydrophobic Pen. Power Block/ Protein Block: Power block was spread over the tissue and wait for 15-20 minutes, blot dry and no washing. Incubation with respective primary antibodies of known dilution for 1-2 hr in humid chamber was done. The slides were washed in PBS buffer, 3 changes of 2 min each. Secondary antibody with polymer HRP was added to section and incubated for 30 min in a humid chamber. The slides were again washed in PBS buffer, 3 changes, 2 min each. Freshly prepared DAB solution was then added to sections for 8-10 min and monitored microscopically for the development of color. The sections were washed in distilled water and counter-staining was done with Harris Haematoxylin for 30 seconds.Sections were dehydrated using ascending grades of alcohol, cleared in xylene and mounted with DPX. Each batch contained a positive control i.e., where staining state is known .

AIMS & OBJECTIVES

a) To study the morphological spectrum of Small Round Cell Tumors in JLN Medical College, Ajmer over a period of two and a half years(Jan 2014 to June 2016). b)To study age wise, sex wise & site wise distribution of Small Round Cell Tumors. c) To study the IHC profile and relevance of IHC in the diagnosis of Small Round Cell Tumors. d) To correlate the histopathological and immunohistochemical diagnosis of various Small Round Cell Tumors. e) To compare the present study with the other studies in India and abroad.

RESULTS

a)Distribution of 76 cases of SRCT according to age and sex

In current study small round cell tumours were more common in males than females. Male to female ratio was 2.04. In age group 6-10 years and 11-15 years small round cell tumors were equal in both males and females. In age group 16-20 years small round cell tumors were more common in females than males. Maximum no. of cases were seen in the age group 56-60 years (13.16%) followed by 51-55 years (10.53%) and 36-40 years (9.21%).

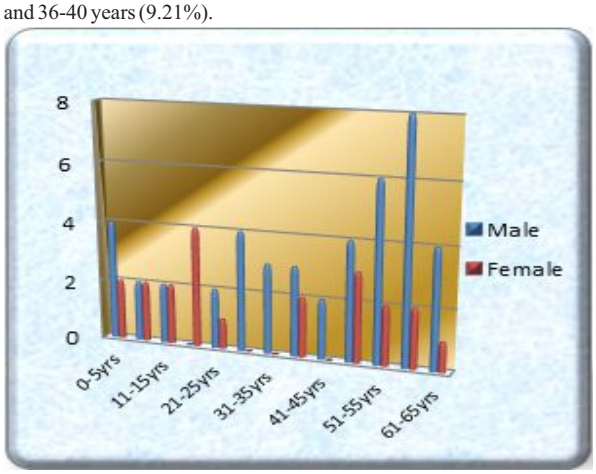

Figure1-Distribution of cases according to age & sex

b)Distribution of 76 cases of SRCT according to site

Majority of cases (36.84%) presented as enlarged lymph nodes, followed by 23.68% of the cases in Head & Neck region,14.47% cases in thorax, 13.16% of the cases in Genitourinary tract, 6.58% of cases in GIT, 3.95% of the cases in soft tissue and only 1.32% of the cases in breast.

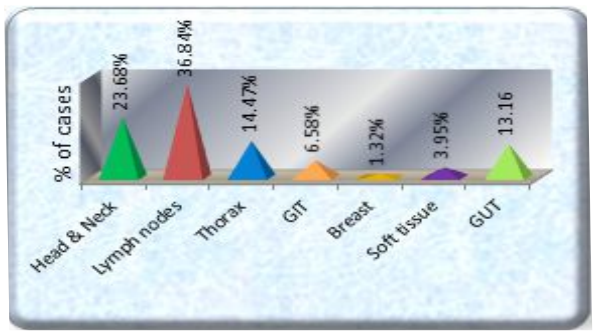

Figure2-Distribution of cases according to site

c)Distribution of 76 cases of SRCT correlating Light microscopy diagnosis & IHC diagnosis

In present study 33% of cases diagnosed as "USRCT" on morphology could be further subtyped on IHC as NHL (45.45%), Undifferentiated RCT(27.27%), Poorly Differentiated Carcinoma (12.12%), Small Cell Anaplastic Carcinoma (6.06%), Nasopharyngeal Carcinoma (3.03%), Medulloblastoma (3.03%), Malignant Nodular Hidradenoma (3.03%). 9 cases(11.84%) remain undifferentiated on IHC due to negative expression of markers that were used in the present study. 16% NHL diagnosed morphologically were confirmed by IHC and were classified as B Cell lymphoma & Lymphoma other than B Cell subtype. Small Cell Anaplastic Carcinoma (87.5%), Retinoblastoma (100%), Medulloblastoma (100%), Seminoma(100%), Dysgerminoma (100), Wilm's Tumour(100%), Rhabdomyosarcoma (100%) diagnosed morphologically were confirmed by IHC. 1 case (12.50%) diagnosed as Small Cell Anaplastic Carcinoma morphologically was reclassified as NHL on basis of IHC.

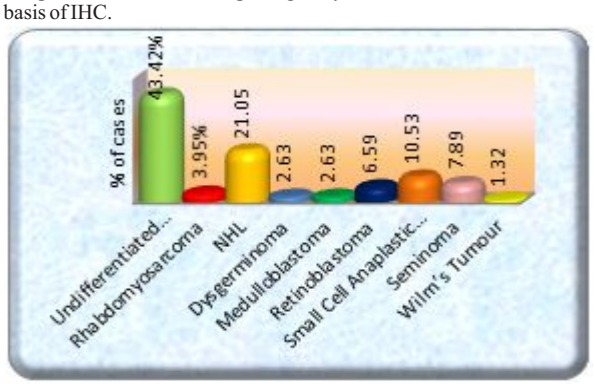

Figure3-Distribution of cases on basis of morphology

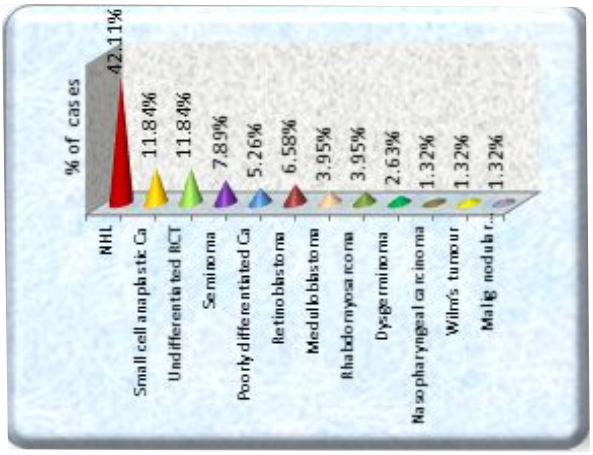

Figure4-Distribution of cases on basis of IHC

Table 1
Distribution of 76 cases of SRCT correlating Light microscopy diagnosis & IHC diagnosis

Histopathological Diagnosis	Final Diagnosis (After IHC Study)	No.	%
Undifferentiated Round Cell Tumour(33)	NHL	15	45.45
	Small Cell Anaplastic Carcinoma	2	6.06
	Poorly Differentiated Carcinoma	4	12.12
	Nasopharyngeal Carcinoma	1	3.03
	Medulloblastoma	1	3.03
	Malignant Nodular Hidradenoma	1	3.03
	Undifferentiated Round Cell Tumour	9	27.27
Small Cell Anaplastic Carcinoma(8)	Small Cell Anaplastic Carcinoma	7	87.50
	NHL	1	12.50
NHL (16)	B Cell Subtype	12	75.00
	Lymphoma other than B Cell Subtype	4	25.00
Seminoma	Seminoma	6	100.00
Retinoblastoma	Retinoblastoma	5	100.00
Medulloblastoma	Medulloblastoma	2	100.00
Dysgerminoma	Dysgerminoma	2	100.00
Rhabdomyosarcoma	Rhabdomyosarcoma	3	100.00
Wilm's Tumour	Wilm's Tumour	1	100.00
TOTAL		76	

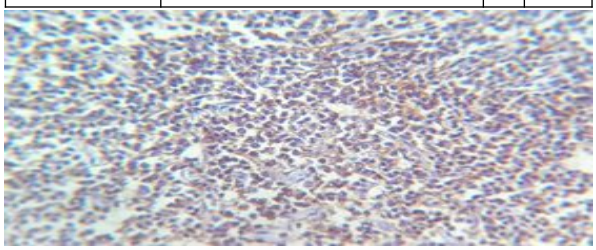

Figure5-NHL- showing immunoreactivity with LCA (under 200x)

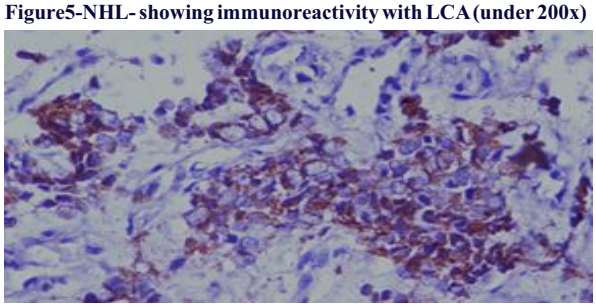

Figure6-Small cell carcinoma lung showing immunoreactivity with PANCK (400X)

DISCUSSION

Differential diagnosis of Small Round Cell Tumours (SRCT) constitutes frequently a diagnostic problem. It appears that biopsy specimens of various neoplasms may present morphology of small round cell tumour(SRCT). Accurate diagnosis of these cancers is essential because the treatment options, responses to therapy and prognosis vary widely depending on the diagnosis. In 80-90% cases of SRCTs, diagnosis can be made by light microscopy coupled with Immunohistochemistry(IHC). Use of ancillary diagnostic techniques has become increasingly important in diagnosis. An initial limited panel of IHC is often the first step towards confirming the differential diagnosis of SRCT. The objective of performing IHC is to recognize cell constituents (antigens) and, consequently, to identify and classify specific cells within a cell population whose morphology is heterogenous or apparently homogenous. The present study includes 76 cases of SRCT–An attempt has been made to study the role of Immunohistochemistry(IHC) in diagnosis of these tumours. In the present study we first applied primary panel of 4 antibodies: PANCK(AE1/AE3), CD45(LCA), S-100 and Desmin. According to their results further IHC markers were applied.

In present study final diagnosis could be achieved in 88.16% cases on the basis of morphology and IHC evaluation. 11.84% cases remained undifferentiated and could not be further subtyped in view of negative expression of markers and unavailability of markers at our institute. Studies by Sajid H. Shah et al[7] & Intisar S.Pity et al[8] reported similar percentage of diagnosis being 87.5% & 88.8% respectively on the basis of morphology and IHC evaluation. The reason for lower % of diagnosis in their studies could be due to higher percentage of undifferentiated tumours in their study. Mandakini M et al[9], Vasudha Bhagat et al[10], Amita Patel et al[11] and J. O. Thomas et al[12] reported higher percentage of cases diagnosed on the basis of morphology and IHC evaluation being 96.25%, 98.65%, 90% and 96% respectively. Lawrence D Cruze (2013) & Bashyal (2011) et al[13] reported higher percentage of diagnosis being 100% combining morphology % IHC. Reason for higher percentage of diagnosis in their studies can be explained by the fact that their study has a small sample size & includes higher percentage of differentiated tumours (e.g. wilms tumour) & site specific tumours (e.g. hepatoblastoma & retinoblastoma).

In the present study Non Hodgkins Lymphoma (42.11%) is the commonest malignancy followed by Small cell anaplastic carcinoma (11.84%). Our study was in concordance with majority of studies by J.O.Thomas, Intisar P.Sity, D Cruze, Bashyal R, Mandakini M, Vasudha Bhagat, AmitaPatel et al which also reported lymphoma to be the commonest tumour being 40%, 16.5%, 44.2%, 52.5%, 32.5%, 24.32% and 47.36% respectively.

In the present study male predominance (67.10%) was seen with male/female ratio being 2.04.Our study was in concordance with various other studies by J.O.Thomas et al, Lawrence D.Cruze et al, Vasudha Bhagat et al & Amita Patel et al which have also reported male predominance with sex ratio being 1.08, 2.90, 1.39 & 1.53 respectively.

Wide age distribution was noted for various SRCTs in most of the studies. Our findings are comparable to most of the other mentioned studies and closest to Vasudha Bhagat et al (2008-2011) & Amita Patel et al (2008-2011). The youngest case was of 4 months of age with final diagnosis of Embryonal Rhabdomyosarcoma, and the oldest patient was 80 yrs of age with diagnosis of Non Hodgkins Lymphoma. In the present study maximum no. of cases (23.69%) were seen in the age group (51-60yrs) followed by 14.47% in the age group (61-70yrs) and 13.16% in the age group (31-40yrs) & (0-10yrs). In the studies by J.O.Thomas et al (1992-1997) & Sajid H.Shah et al(1995-1996) age distribution was found to be 6 mth-14 yrs & 1-15 yrs, reason probably being inclusion of cases of <15 yrs age only.

CONCLUSION

Small Round Cell Tumours poses a diagnostic challenge to the pathologist as majority of tumours are undifferentiated and they have similar morphological appearance (Round cells with hyperchromatic nuclei and scanty cytoplasm) on H & E stained sections. IHC is a very useful diagnostic tool in differential diagnosis of small round cell tumours in conjunction with morphological features and clinical parameters. Although use of IHC panel is expensive but considering

the specific therapeutic protocol of small round cell tumours, accurate and timely diagnosis is important. A panel of antibodies should be ideally put up for every case for diagnosis of these tumours and an awareness of reactivity patterns, sensitivity, specificity and potential diagnostic pitfalls is essential.

NHL exclusively express CD45, so CD45 is a reliable marker for diagnosis of lymphoma. B cell NHL express CD79a, so CD79a helps in further subtyping NHL and is a specific B-cell marker. Small cell anaplastic carcinoma & nasopharyngeal carcinoma express PANCK, so PANCK is a reliable marker for diagnosis of these malignancies. Embryonal Rhabdomyosarcoma express desmin. It is a reliable marker for diagnosis for these malignancies. Wilms Tumour and Seminoma express WT1 & PLAP respectively, & can be diagnosed in combination of classical histological finding along with clinical presentation. Dysgerminoma express vimentin thus vimentin is a reliable marker to diagnose dysgerminoma. Retinoblastoma express NSE, however, NSE is a non specific marker for retinoblastoma.

In summary, a panel of monoclonal antibodies (including both positive and negative) is recommended for the diagnosis of small round cell tumours. The use of immunomarkers requires standardization & strict protocol laid down for every step of the procedure. Immunohistochemistry should always be used as an adjunct to the morphology, to avoid an erroneous diagnosis. In recent years, a better understanding of the molecular genetic studies of these tumours allows a molecular testing as a further valuable tool for arriving at a definitive diagnosis in the questionable cases.

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CONFLICT OF INTEREST- None.

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