Original Research Article

Spectrum of round cell tumors in North-Western region of India

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ABSTRACT

Introduction: Round cell tumors are poorly differentiated tumors. Immunohistochemistry is a useful and cost effective approach for differentiation of Malignant round cell tumors. NonHodgkin’s Lymphoma and Ewing Sarcoma are most commonly encountered tumors among them.

Aim: (1) For categorization of round cell tumors. (2) To Study the occurrence and distribution of tumors in different age groups.

Materials and Methods: We studied 141 cases over a period of one year (January 2017 to December 2017). Formalin fixed, paraffin embedded sections of tumors diagnosed on small biopsies as well as resected specimens were taken. Immunohistochemistry was done on cases which were reported as malignant round cell tumors on routine HPE.

Results: Non Hodgkin’s lymphoma was the commonest among these small round cell tumors comprising 51.77% (73 cases). The next most common tumor was Ewing’s Sarcoma/PNET which had 28 (19.86%) cases. Other were small cell carcinoma of Lung (9.93%), Neuroblastoma, (4.26%) Medulloblastoma (3.54%), Rhabdomyosarcoma (2.84%) and Wilm’s tumors (2.84%). We found 18 cases of Neuroendocrine tumors of GIT in which two cases were small cell variant of neuroendocrine carcinoma of GIT (1.42%).

Conclusion: Clinical details, morphology and Immunohistochemistry play a vital role in differentiating malignant round cell tumors.

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1. Introduction

Pathologists commonly encounter a myriad of morphological patterns in the biopsies that they see in their routine clinical practice. Tumors which show differentiation towards their native cell of origin are easy to diagnose. Poor differentiation leads to a diagnostic dilemma. The term malignant round cell tumor or small round cell tumor or small round blue cell tumor (SRBCT) applies to a group of highly aggressive tumors composed of patternless sheets of undifferentiated, small and monotonous cells with increased nuclear-cytoplasmic ratio. Most of the times, light microscopy on Hematoxylin and Eosin (H&E) stain fails to diagnose malignant round cell tumors. The diagnosis of SRBCT is solely based on IHC and cytogenetics. The tumors commonly included in this category are NHL, Ewing’s/PNET, neuroblastoma, small cell carcinoma, rhabdomyosarcoma and medulloblastoma. Other tumors that sometimes present as round cell tumors are Wilm’s tumor, Desmoplastic Small Round Cell tumor (DSRCT). The list can be expanded if we consider site specific round cell tumors.

2. Materials and Methods

We studied 141 cases submitted to the department of Pathology, SMS Medical college and attached Hospitals, Jaipur over a period of one year (January 2017 to December 2017). Formalin fixed, paraffin embedded sections of tumors diagnosed on small biopsies as well as resected
study of Bashyal R et al. and Ahmed Z et al. also showed lymphoma as the most common tumor type (52.5% and 65.30% respectively) in round cell tumors. On performing IHC, tumor cells showed cytoplasmic and membranous immunoreactivity for CD45 in all cases of Non Hodgkin’s Lymphomas. The most common occurring NHL was DLBCL. Negative staining for Pan-Cytokeratin (Pan-CK) differentiated these tumors from carcinomas. They were also negative for specific markers of other round cell tumors. Further categorization of Non Hodgkin’s Lymphoma was done using second IHC panel that included CD20, CD3, CD10, Bcl2, Bcl6, CD5, CD23, MUM-1 and Ki67. Two cases of Burkitt’s lymphoma were diagnosed with clinical details and markers as CD 20, CD 10 and BCL-6 positivity. MIB score were 95% and 98% respectively.

Second most common category of round cell tumour was Ewing Sarcoma/PNET. Ewing Sarcoma usually arises from the diaphysis and metaphyseal region of long bones, but can affect any bony site. In present study, CD99 positivity was present in 24 out of 28 cases of Ewing Sarcoma. Cases negative for CD99 were confirmed as Ewing Sarcoma/ PNET because nuclear expression of NX2.2 and FLI1. In our study, Ewing Sarcoma most commonly occurred in less than 30 years of age. Four patients were age above 30 years of age. Male to Female ratio was 4:3. Folpe AL et al study showed CD99 positivity in all cases of Ewing Sarcoma and T-Cell lymphoblastic lymphoma, while only 80% B cell lymphoblastic lymphoma cases showed CD99 expression. Labeling of Lymphoblastic Lymphoma is a difficult task because of its overlapping morphological and immunological features with Ewing Sarcoma. Lymphoblastic Lymphoma shows positivity for CD99 and CD45 both.

Again, we had three more cases received as core needle biopsy, diagnosis changed after IHC. A 19 years old male patient, presented as chest wall mass, reported as Ewing Sarcoma/ Liposarcoma on histopathology. Section showed more fat cells, and fibrous tissue with crushing artifacts on H and E examination. On performing IHC, tumor cells were positive for CD99, NKX2.2 and FLI1 and at the same time negative for S100, CD34, Myogenin and EMA, finally labelled as Ewing Sarcoma. Another case, an 18-month-old patient diagnosed as Ewing Sarcoma/PNET, came with differential diagnosis of Neuroblastoma because of retroperitoneal location and histomorphology. Similarly, a 5 year old female child having vertebral mass diagnosed as PNET on IHC with CD99, FLI1, CD56, Vimentin and EMA positivity. This tumor was negative for CD34,NSE, OCT 3 and PLAP.

Study done by Mudassar M et al. also described Lymphoma (40.6%) as commonest among round cell tumors, followed by Ewing Sarcoma/PNET (9.5%).

In our study, only two cases were diagnosed as small cell variant of neuroendocrine carcinoma of gastrointestinal tract. Neuroendocrine carcinoma occur in various sites

3. Results

Round cell tumors are characterised by scant cytoplasm and dark hyperchromatic nuclei. These tumors show characteristics microscopic appearance of small round cells that stain blue on routine Haematoxylin and Eosin stain. Instead of similar morphology, they are different genetically and biologically. Because light microscopy alone cannot categorize them, ancillary techniques such as IHC, electron microscopy, cytogenetics, molecular profiling etc are required for separating these tumors from one another. IHC is easy to setup and cost effective from others. A panel of antibodies should be employed to give a specific diagnosis to rule out other lesions. Patients in this study belonged to all age groups, ranging from 8 months to 71 years. Most of the cases were of age more than 30 years (60.28%). Of the 141 cases, 98 (96.50%) were males and 43 (30.50%) were females. Non Hodgin’s lymphoma was the commonest among these small round cell tumors comprising 51.77% (73 cases). The next most common tumor was Ewing’s Sarcoma/PNET which had 28 (19.86%) cases. Table 1 shows the frequency of cases in our study. Out of total 73 cases of NHL, 37 were Nodal and 36 from Extra nodal sites. In extra nodal lymphoma, the common sites were nasopharyngeal and GIT. The other sites were parotid gland, cutaneous, soft tissue, testes, ovaries and orbital region for Lymphoma.

4. Discussion

Differential diagnosis of SRBCT includes varied group of tumors, treatment and management of each categories is different. So, a careful examination of H & E sections in an appropriate clinicoradiological background is required before using IHC for conclusive diagnosis. Histological diagnosis of most of the cases can be made by use of extensive panel of antibodies in malignant undifferentiated neoplasms.

In this study, Lymphomas were the most common entity among all other round cell neoplasms (51.77%). Study of Bashyal R et al. and Ahmed Z et al. also

Specimens were taken. Cases diagnosed as Malignant round cell tumors on routine histopathological examination were included in this study. Immunohistochemistry was done using Peroxidase-antiperoxidase method with heat induced antigen retrieval technique. Ready-to-use primary antibodies of Biocare Medical were used. Positive and negative controls were used as per protocol. The panel selection was done based on histomorphological features and relevant clinical information. Accordingly the first broad panel of antibodies used included pancytokeratin (Pan-CK), LCA, vimentin, S100, neuroendocrine markers, CD99 and desmin. Second panel of antibodies were applied according to the results of the first panel.
Fig. 1: Non Hodgkin’s lymphoma (HE 100X): Lymphoma cell are CD20 positive (a1), CD3 negative (a2); (b) Ewing sarcoma) HE400X): tumor cells are positive for (b1), (b2) NKX2.2, CD99 respectively; (c) Rhabdomyosarcoma (HE 400X): tumor cells are positive for (c1), (c2) Demin, Myogenin respectively; (d) Medulloblastoma (HE 100X): Tumor cells are (d1) GFAP negative, (d2) Synptophysin positive
Fig. 2: Small cell carcinoma (HE 100X): Tumor cells are positive for (e1), (e2) Synaptophysin, Chromagranin; (e3) TTF-1 negative

Table 1: Final diagnosis of cases after IHC

| Diagnosis                                      | Total cases | Percentage |
|------------------------------------------------|-------------|------------|
| NHL                                            | 73          | 46.50      |
| Ewing’s tumour/PNET                            | 28          | 17.83      |
| Small cell Carcinoma lung                      | 14          | 8.92       |
| Neuroendocrine carcinoma GIT                   | 18          | 11.46      |
| Neuroblastoma                                  | 6           | 3.82       |
| Medulloblastoma                                | 5           | 3.18       |
| Rhabdomyosarcoma                               | 4           | 2.55       |
| Wilm’s Tumour                                  | 4           | 2.55       |
| CNS PNET                                       | 1           | 0.64       |
| Lung small cell anaplastic carcinoma           | 1           | 0.64       |
| Merkel cell Carcinoma                          | 1           | 0.64       |
| Inconclusive                                   | 2           | 1.27       |
| Total                                          | 157         |            |
Table 2: Age wise distribution of round cell tumors

| Tumor type                        | <=5 | 6-15 | 16-30 | 31-60 | >60 |
|-----------------------------------|-----|------|-------|-------|-----|
| NHL                               | 1   | 3    | 9     | 29    | 31  |
| Ewing’s tumour/PNET               | 1   | 4    | 19    | 2     | 2   |
| Small cell Carcinoma lung         | 1   | -    | -     | 6     | 7   |
| Neuroendocrine carcinoma GIT      | -   | 2    | 2     | 8     | 6   |
| Neuroblastoma                     | 3   | 1    | -     | 2     | -   |
| Medulloblastoma                   | -   | 3    | -     | 2     | -   |
| Rhabdomyosarcoma                  | 1   | 3    | -     | -     | -   |
| Wilm’s Tumour                     | 4   | -    | -     | -     | -   |
| CNS PNET                          | 1   | -    | -     | -     | -   |
| Lung small cell anaplastic carcinoma | -  | -    | -     | 1     | -   |
| Merkel cell Carcinoma             | -   | -    | -     | 1     | -   |
| Inconclusive                      | -   | 2    | -     | -     | -   |
| Total                             | 12  | 18   | 30    | 51    | 46  |
such as brain, Mediastinal and parapharangeal locations. In our study, we didn’t find neuroendocrine carcinoma from other sites except Lung and GIT. In these, one liver core biopsy were reported as poorly differentiated carcinoma with crushing artifacts. On IHC, tumor cells were negative for HepPar 1 and TTF1. Tumors cells were positive for Pancytokeratin, CDX2, CD56 and Synaptophysin, so reported as Metastatic Neuroendocrine Carcinoma. Patient had a mass lesion in small intestine on CT scan so probability of GI origin was suggested.

In present study, we had 14 cases of primary small cell Lung carcinoma and Metastatic Small cell carcinoma of Lung. All cases except one were above 40 years of age. These cases were CD56 positive (92%), Synaptophysin positive (90%), TTF1 positive (60%) and Pancytokeratin positivity (91%). Metastatic small cell carcinoma of lung from other sites diagnosed with exclusion criteria, showed no expression of markers such as LCA, S100, vimentin, and CDX2. David Dabbs showed variable sensitivity for neuroendocrine markers like CD56, Synaptophysin, Chromogranin and NSE which were 95%, 80%, 85% and 82% respectively.

Similar studies which were done by Tadashi Terada showed synaptophysin positivity (85%) and chromogranin positivity (62%) in the carcinoids. According to David Dabb’s, many SCLCs don’t express Chromogranin A, although the expression is proportional to cytoplasmic granules of neoplastic cells.

Two cervical lymph node biopsies were primarily reported as Lymphoproliferative disorder on histopathology, however on IHC (LCA, CD99, CK, CD56, TTF and Synaptophysin), they were confirmed as metastatic small cell carcinoma of Lung.

Six cases were diagnosed as Neuroblastoma. Four cases were of age below 15 years while two cases reported as Olfactory neuroblastomas were above 40 years. All of our cases of neuroblastoma were positive for CD56 (100%), three cases showed NSE positivity, two cases were NF, Chromogranin and Synaptophysin positivity. All cases showed LCA, Tdt, PanCK, Vimentin and CD 99 negativity.
David Dabbs showed synaptophysin positivity in almost every cases of olfactory neuroblastoma.10 Five cases with posterior fossa SOL, were reported as Medulloblastoma. They were positive for CD99, Synaptophysin and GFAP. Three cases were less than 10 years of age. Four cases were reported as Rhabdomyosarcoma. These cases aged less than 15 years and all were males. Amongst these, three were from Head and Neck region and one was from abdominal wall. All were positive for Vimentin, Desmin and Myogenin, while negative for LCA and CD 99. One case showed dim positivity for pancytokeratin. Antibodies to desmin, myogenin and MyoD1 are useful markers to differentiate Rhabdomyosarcomas from other small round cell tumors.11

Four cases were reported as Wilm’s Tumor. The patients were in age range of 1 to 5 years. All cases showed diffuse strong to moderate nuclear positivity of WT1 in blastemal and epithelial component. One case showed CD56 and another for NSE positivity. Study done by S goyal et al showed two groups of Wilm’s tumor patients. One group showed predominantly diffuse strong to moderate blastemal (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic and other group comprised eight (75%) and epithelial (80%) positivity which was both nuclear and cytoplasmic. 3 They found variability in the intensity of WT1 staining in the different components of the same patient and among the tumors having the same stage.12,13

5. Conclusion
Categorisation of Malignant round cell tumors is mandatory. Clinical details, morphology and IHC play a vital role in differentiating malignant round cell tumors. In malignant round cell tumors, core needle biopsies are the main limitation. Because of amount of tissue, quality of tissue and processing artifacts, diagnosis can not be possible without IHC or cytogenetics. On ladder of diagnostic tool, IHC is a cost effective and less time consuming in resource poor regions. The purpose of categorisation is to guide clinicians for treatment modalities whether patient requires chemotherapy, radiotherapy, resection or any specific targeted therapy for round cell tumors of different origin. IHC also helps to identify the risk of recurrence and fatality rate of tumor. The wisely used panel with clinicopathological correlation give ultimate diagnosis of undifferentiated malignant round cell tumors.

6. Source of Funding
None.

7. Conflict of Interest
The authors declare that there is no conflict of interest.

References
1. Gregario A, Corrias MV, Castriconi R, Dondero A, Mosconi M, Gambini C. Small round blue cell tumors’ Diagnostic and prognostic usefulness of surface molecule. Histopathology. 2008;53(1):73–80.
2. Ahmed Z, Azad NS, Bhurgari Y, Ahmed R, Kayani N, Perviz S, et al. Significance of immunohistochemistry in accurate characterization of malignant tumors. J Ayub Med Coll Abbottabad. 2006;18(2):38–43.
3. Bashyal R, Pathak TB, Shrestha S, Pun CB, Banstola S, Neupane S, et al. Role of immunohistochemistry in the diagnosis of malignant small round cell tumors. J Pathol Nepal. 2011;1:87–91.
doi:10.3126/jpn.v11i2.3398.
4. Folpe AL, Goldblum JR, Rubin BP, Shehata BM, Liu W, Tos APD, et al. Morphologic and immunophenotypic diversity in Ewing family tumors: a study of 66 genetically confirmed cases. Am J Surg Pathol. 2005;29(8):1025–33.
5. Ozdemirli M, Smith JCF, Hartmann DP, Azumi N, Miettinen M. Differentiating Lymphoblastic Lymphoma and Ewing’s Sarcoma: Lymphocyte Markers and Gene Rearrangement. Modern Pathol. 2001;14(11):1175–82.
doi:10.1097/00004098-200105000-00009.
6. Madassar M, Baloch FA, Hameed S, Zubair S, Sohail SK, Kamran M. Use of Immunohistochemistry in the differential diagnosis of Small Round Blue cell tumors. Prof Med J. 2020;27(08):1728–36.
doi:10.29309/pmpj.2020.27.08.401.
7. D’ercole L, Dutta R, Rao S, Anuradha R, Varadarajan S, Kuruvilla S. Role of Immunohistochemistry in the Analysis of the Spectrum of Small Round Cell Tumours at a Tertiary Care Centre. J Clin Diagn Res. 2013;7(7):1377–82.
8. Hammar SP, Dacic S. Immunohistochemistry of Lung and Pleural Neoplasms. In: Dabbs DJ, editor. Diagnostic immunohistochemistry, theranostic and genomic applications. Philadelphia: Saunders Elsevier; 2014. p. 402.
9. Terada T. Carcinoid Tumors of Digestive Organs: a Clinicopathologic Study of 13 Cases. Gastroenterol Res. 2009;2(1):35–7.
10. Hunt JL. Diagnostic immunohistochemistry, theranostic and genomic applications. In: Dabbs DJ, editor. Diagnostic immunohistochemistry, theranostic and genomic applications. Philadelphia: Saunders Elsevier; 2010. p. 264.
11. Wang NP, Marx J, McNutt MA. Expression of myogenic regulatory proteins (myogenin and MyoD1) in small blue round cell tumors of childhood. Am J Pathol. 1995;147:1799–1810.
12. Mishra K, Goyal S, Sarkar U, Sharma S, Kumar A. Diagnostic utility of Wilms’ tumour-1 protein (WT-1) immunostaining in paediatric renal tumours. Indian J Med Res. 2016;143(7):59–67.
doi:10.4103/0019-5576.169176.
13. Ramani P, Cowell JK. The expression pattern of Wilms’ tumour gene (WT1) product in normal tissues and paediatric renal tumors. J Pathol. 1996;179(2):162–8.
doi:10.1002/(sici)1096-909x(199601)179:2<162::aid-path345>3.0.co;2-u.

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