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Study of Paediatric Small Blue Round Cell Tumors with Immunohistochemical Correlation

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Abstract: *Small Blue Round Cell Tumors (SBRCT) include a heterogenous group of neoplasms most commonly seen in paediatric age group. With the advent of specific treatment protocols, pathologist must thrive to categorise SBRCT into a defined diagnosis. 56 cases of SBRCT were studied over a period of two years and differentiated histologically by Fine needle aspiration cytology (FNAC) and Histopathological examination (HPE). In some cases accurate diagnosis was possible by special stains and Immunohistochemistry (IHC)*

Keywords: *Small Blue Round Cell Tumors (SBRCT), Fine needle aspiration cytology (FNAC), Histopathological examination (HPE), Immunohistochemistry (IHC)*

I. Introduction

Small Blue Round Cell Tumors (SBRCT) is the name given to a group of malignant neoplasms that occur mostly in the paediatric age group (upto 14 Yrs.) These tumors show characteristic appearance under the microscope, consisting of small round cells that stain blue on routine Haematoxylin and Eosin staining. (1). Incidence of SBRCT is on rise all over the world (2) Over 60% of all childhood SBRCT are now curable (3)

Fortunately a variety of techniques such as special stains, immunohistochemistry, electronmicroscopy, cytogenetics, molecular genetics, insituhybridization and insitu Polymerase Chain Reaction are now available which help in the study of various undifferentiated SBRCT that help in modern oncotherapy.

II. Aims And Objectives

Study of Small blue round cell tumors with cytohistopathological correlation taking aid of clinical, radiological & Immunohistochemical findings.

III. Material And Methods

This study comprises of 56 cases of SBRCT in chidren upto the age of 14 Years, over a period of two years. FNAC, HPE and Squash cytology (in a few cases) were done using Haematoxylin and Eosin stain. Special stains like PAS and reticulin stains were done for few cases. Immunohistochemistry was done for few tumors.

IV. Results And Observation

The total number of cases included in this study are 56 over a period of two years. Complete details including age, sex and clinical presentation are available for almost all the cases. Clinical, radiological, cytological, gross, histological features and immunohistochemical findings for a few small blue round cell tumors are analysed according to the type of tumor.

TABLE-1; Frequency of Varios Small Blue Round Cell Tumors

Tumor	Total No	Percentage of SBRCT
Lymphomas	16	29%
Nephroblastomas	15	27%
Neuroblastomas	7	12%
Rhabdomyosarcomas	8	14%
Ewing's sarcoma	5	9%
Hepatoblastoma	2	4%
Medulloblastoma	1	2%
Pancreatoblastoma	1	2%
Ependymoma	1	2%
Total	56	100%

Table-2; Relative Frequency of Tumors in Various Age Groups and Sexes

Tumor	Less than 1 year		1-4 years		5-9 years		10-14 years		Total
	M	F	M	F	M	F	M	F	
SBRCT									
Nephroblastoma	3	4	4	3	1	0	0	0	15
Neuroblastoma	0	1	2	2	1	0	1	0	7

Lymphomas	0	0	0	1	7	1	6	1	16
Rhabdomyosarcoma	0	0	1	1	3	1	2	0	8
Ewing's sarcoma	0	0	0	1	0	2	1	1	5
Hepatoblastoma	1	1	0	0	0	0	0	0	2
Pancreatoblastoma	0	0	0	0	1	0	0	0	1
Medulloblastoma	0	0	0	0	0	1	0	0	1
Ependymoma	0	0	0	0	0	1	0	0	1
Total	4	6	7	8	13	5	11	2	56

TABLE-3; Correlation of cytological & histopathological Diagnosis

No.of cases-34	CYTOLOGICAL DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS
4	Neuroblastoma	Neuroblastoma
1	Neuroblastoma	1.Ewings' sarcoma 2.Neuroblastoma
1	Neuroblastoma	Secondary deposit of Blastoma; possibly 1.Neuroblastoma, 2.Nephroblastoma
2	Blastoma	Neuroblastoma
4	Nephroblastoma	Nephroblastoma
1	Blastoma	Nephroblastoma
1	Nephroblastoma	Extra renal wilms' tumor
2	Hepatoblastoma	Hepatoblastoma
1	Undifferentiated sarcoma	Embryonal Rhabdomyosarcoma
4	Ewing's sarcoma	Ewing's sarcoma
1	Lymphoma	Embryonal rhabdomyosarcoma
2	Rhabdomyo sarcoma	Embryonal Rhabdomyosarcoma
2	Lymphoma	Hodgkins Lymphoma- Mixed cellularity
1	Lymphoma	Hodgkin's Lymphoma- Lymphocytic predominant
4	Lymphoma	NHL-Large cell Lymphoma
1	Lymphoma	Burkitt's lymphoma
1	Medulloblastoma	Medulloblastoma
1	Ependymoma	Ependymoma

56 SBRCT were studied out of which lymphomas (16) and nephroblastomas (15) constitute the majority. Two Hepatoblastomas, one Medulloblastoma, one Ependymoma and one Pancreatoblastoma were also among those studied (Table-1).

Nephroblastomas, Neuroblastomas and Hepatoblastomas diagnosed in the age group of less than 5 years whereas most of other tumors diagnosed after 5 years. Sex incidence was almost equal except in cases of lymphomas with male preponderance (Table-2).

FNAC was done for a total of 41 cases. For 7 cases only cytological examination could be done as patients were unfit for surgery. Hense 34 cases could be studied both by cytology and histopathology. Of 34 cases studied cytologically 30 (88%) cases correlated with histopathological diagnosis (Table-3). Three cases reported on FNAC as Blastoma and suggested the possibility of neuroblastoma in two cases and Nephroblastoma in one case were confirmed by HPE.

Immunohistochemistry was done in 6 cases of doubtful diagnosis (Table-4).

Table-4; Small Blue Round Cell Tumors-Immunohistochemistry

SlNo	Sex/Age	Clinical Presentation	Cytological Diagnosis	Histopathological Diagnosis	Immunohisto-chemistry & Diagnosis
1.	M/9 Yr.	Mass in abdomen	Neuroblastoma	Neuroblastoma	NSE-Diffuse positive; **Neuroblastoma**
2.	F/3 Yr.	Mass in Abdomen	Neuroblastoma	Secondary deposit of Blastoma possibly 1.Neuroblastoma 2.Nephroblastoma	NSE-Focal positive. **Neuroblastoma**
3.	F/1Yr.	Swelling on ant.aspect of middle one third of thigh	Neuroblastoma	s/o SBRCT possibly 1.Ewing's sarcoma. 2.Neuroblastoma.	NSE-Focal positive CD99-Diffuse positive PAS positive: **Ewing's sarcoma**
4.	F/6	Rt.parietal space occupying lesion	**NA**	Medulloblastoma	NSE; few cells positive; **Medulloblastoma**
5.	M/8Yr.	Rectal polyp	**NA**	NHL-large cell type with surface mucosal ulceration	LCA-positive CD3-focal positive. CD20-large cells positive. **NHL-large cell type**
6.	M/7	Cervical lymphadenopathy	Lymphoma	1.NHL-large cell type. 2.Secondary deposit	Cytokeratin negative LCA positive. **NHL-large cell type**

V. Discussion

Those days when treatment of childhood SBRCT was simply a matter of resecting as much of the tumor as possible, followed by radiotherapy to the primary site, the precise histological diagnosis was of little importance. With advances in three main forms of treatment-surgery, radiotherapy and chemotherapy-exact classification of these lesions have become of paramount importance.

The present study includes a total number of 56 SBRCT and emphasis was laid in trying to classify these into specific tumors instead of labeling them as "small blue round cell tumors" as we have been doing before. Only 6 cases were submitted for immunohistochemistry where we definitely could not come to any conclusion of the tumor type by histopathological examination (Table-4).

In the small blue round cell tumors Nephroblastomas (Wilms' tumor) and it's variants were 15 cases constituting 27% of small blue round cell tumors . 14 cases (93%) were seen in children below the age of 4 years (4), the youngest was 5 months old and the oldest was 6 years old. Majority of cases presented with mass per abdomen. One case was seen as an extrarenal mass being attached to the inferior surface of kidney and arising from a small renal duplication.

FNAC was done for 8 cases of Nephroblastoma and we did suggest the possibility of a nephroblastoma in all cases (5). 2 cases were unfit for surgery and hense we could not receive biopsy specimen. We received 7 cases of biopsy specimens directly without prior FNAC.

Microscopically, ten cases were classical triphasic wilms' tumor with blastemal, stromal and epithelial elements (favorable histology-ref.6). The predominant component was mentioned in our report. One case was a biphasic Wilms' with epithelial predominance and the other component being stromal. (7). Three multicystic lesions were seen microscopically to consist of islands of blastemal tissue inside the stroma surrounding the cysts and hence labeled as cystic partially differentiated nephroblastoma (Multicystic nephroma) (7) . No capsular or vascular infiltration was seen in all the cases. There was no infiltration into ureter.

A single case of extrarenal Wilms' tumor showed classical triphasic pattern and I.V.P. after the surgery showed both kidneys with normal excretion. The tumor tissue studied showed normal renal tissue and ureter. Extrarenal Wilms' are assumed to have developed in embryonic remnants or rests such as ectopic metanephrogenic blastema in a small renal duplication (8).

One case of Wilms' tumor showed features of anaplasia- large round to oval hyperchromatic nuclei and abnormal mitotic figures in both blastemal and stromal component (unfavorable histology-ref 6). It also showed tumor giant cells and Rhabdomyoblastic cells against a myxoid background with extensive areas of necrosis and hemorrhages. vascular invasion and capsular infiltration by tumor cells was also seen.

A total no of 7 cases of neuroblastomas were studied constituting 12% of small blue round cell tumors . 5 cases were seen in children below the age of 5 years (75%) which is consistent with literature (9). In our study the youngest case was one year old and the oldest was 10 year old. 6 cases presented as an abdominal mass and one case as swelling middle one third of thigh.

Cytologically a diagnosis of neuroblastoma was made in five cases and two cases were reported as ' Blastoma' and suggested the possibility of Neuroblastoma.

On HPE four cases were confirmed as neuroblastomas and other three cases with doubtful diagnosis were submitted for immunohistochemistry. Two of these cases were positive for Neuron Specific Enolase (NSE) and hence reported as neuroblastomas. But one case showed only scant focal postivity and was focally positive for vimentin and PAS and diffusely positive for CD99. This was diagnosed as an Ewing's sarcoma and the site being middle one third of thigh (Table-4).

The classification of neuroblastoma is known to be intimately related to grading and prognostic features (8). The grading system followed by Joshi et al, 1992 was shown to relate to prognosis better than the degree of differentiation or age of patient (10). This grading system was based on mitotic count and presence of calcification.

Grading system proposed by Joshi et al; 1992 (10)

Grade	Description	Age	Prognostic group
I	Low mitotic rate (≤10/10HPF) and calcification	Any	Low risk
II	Low mitotic rate or calcification	≤ 1 yr	Low risk
III	High mitotic rate (>10/10/HPF) and no calcification	Any	High risk

Grading in our study was as follows:

Grade II; 4 cases.

Grade III; 2 cases

A total number of 8 cases of rhabdomyosarcoma were studied constituting about 14% of small blue round cell tumors . 4 cases (50%) were younger than 5 years of age. Youngest case was found to be 1 ½ year old male child and the oldest case reported was 14 year old male child.

Clinically most commonly seen in head and neck (50%), followed by extremities (25%) and abdomen (25%).

FNAC done in five cases. four cases were diagnosed as Embryonal Rhabdomyosarcoma (Fig.1) and one case was diagnosed as Undifferentiared sarcoma. Two cases were unfit for surgery.

On HPE all three cases confirmed as Embryonal Rhabdomyosarcoma (Fig.2). One case reported as Lymphoma on FNAC was confirmed as Embryonal Rhabdomyosarcoma and we received two more case for HPE directly which were diagnosed as Embryonal Rhabdomyosarcoma.

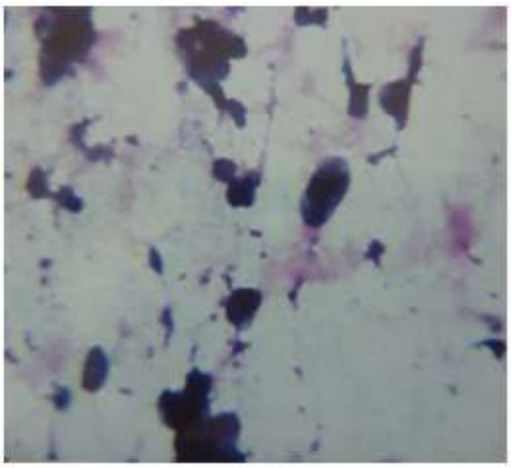

Fig-1: ERMS; FNAC

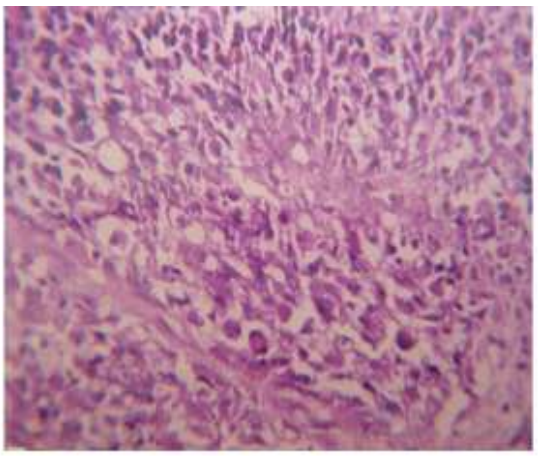

Fig-2; ERMS; HPE

Schmidt et al (11) distinguished least or primitive differentiated (less than 10% rhabdomyoblasts), better or intermediate differentiated (10-50%) and well differentiated (More than 50%) forms of embryonal rhabdomyosarcoma.

Grading is based on differentiation (assessed by the presence of rhabdomyoblasts), maturation (degree and pattern), proliferation (based on mitotic activity and necrosis), architecture (characterised by the presence of myxoid areas and fibrous septa) and it correlates well with clinical course. In their study (8) three features which correlated with a favorable outcome in all types of rhabdomyosarcomas were a high degree of maturation, absence of tumor necrosis and no fibrous septa.

In our study grading done in 6 cases according to Schimidt et al (11);

Intermediate differentiated; 4 cases

Least differentiated ; 2 cases

In our study 16 cases of lymphomas constituting 29% of SBRCT were noted. The youngest case reported was 4 years old, oldest being 14 years. The majority of tumors were seen above the age of 5 years in comparison to other SBRCT. 10 were Non-Hodgkin's lymphoma and 6 were Hodgkin's.

Non Hodgkin's Lymphoma presented with cervical lymphadenopathy and in a few with generalized lympdadenopathy and hepatosplenomegaly. One case presented as rectal polyp with bleeding per rectum.

FNAC done in twelve cases. Cytologically all cases diagnosed as lymphoma. In one case peripheral smear and bone marrow aspiration showed picture of acute lymphoblastic leukemia (Non-Hodgkins lymphoma with terminal leukemic phase). Three cases were unfit for surgery. We received biopsy specimens for five cases without prior FNAC.

Microscopically majority of the cases including rectal polyp showed a diffuse large cell lymphoma (fig.3). One case in a 10 year old male child showed features of a Burkitt's lymphoma. One case was diagnosed as Embryonal Rhabdomyosarcoma (Table-3).

Two cases were submitted for immunohistochemistry, where the differential diagnosis was a secondary deposit from an epithelial malignancy. Both the cases were positive for Leucocyte common antigen (LCA or CD 45) and negative for pancytokeratin confirming their lymphoid lineage. In the case of rectal polyp LCA positive(Fig.4), CD3 focal positive and CD 20 diffuse postive. Hence the features were consistent with a Non-Hodgkin's lymphoma, large cell B-cell type with few reactive T-cells in between.(8) . In paediatric practice virtually all NHLs are of high grade and most are extranodal and non follicular but in our study most of them were nodal.

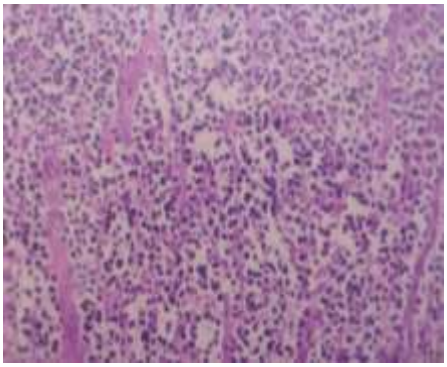

Fig-3; RECTAL POLYP-NHL; HPE

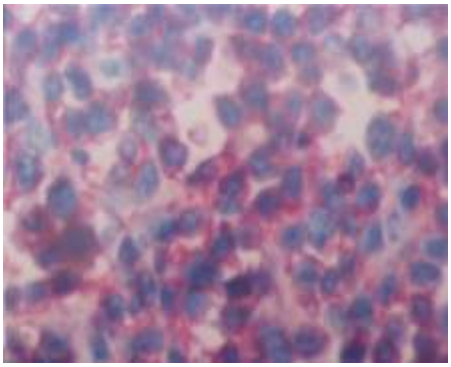

Fig-4; RECTAL POLYP-NHL; LCA

Six cases of Hodgkin's disease were studied, two being lymphocytic predominant type, three being mixed cellularity type and one case being nodular sclerosis variant. The microscopic picture was comparatively easier to diagnose in Hodgkin's disease when compared to Non-Hodgkin's.

A total of 5 cases of Ewing's sarcoma were studied constituting 9% of small blue round cell tumors seen in the age group of 10-13 years which is consistent with the literature (12).

Cytologically, a diagnosis of Ewing's sarcoma was made and confirmed microscopically. PAS stain was positive in 4 cases (Fig.5,6,**7**).

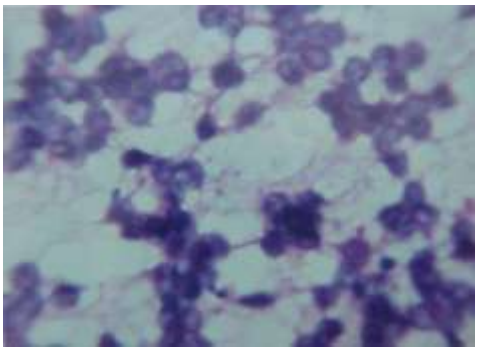

Fig-5; Ewing's Sarcoma; FNAC

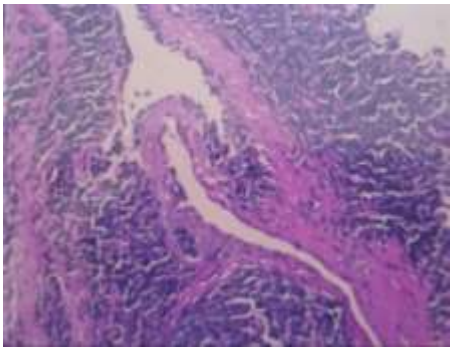

Fig-6; Ewing's Sarcoma; HPE

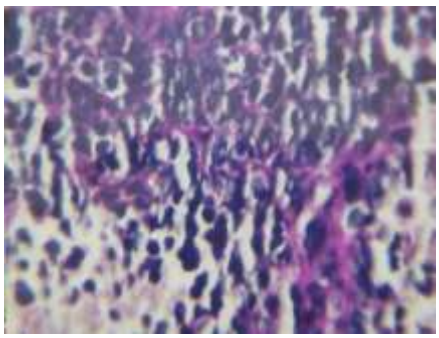

Fig-7; Ewing's Sarcoma; PAS

In one case which was in the middle of thigh we had a differential diagnosis of a neuroblastoma secondaries or an Ewing's sarcoma. Hence we submitted the case for immunohistochemistry, which showed patchy positivity for NSE , positivity for vimentin, PAS and was also positive for CD99, a diagnosis of Ewing's sarcoma was concluded (Table-4)

Of the cases we have studied three cases were localised tumors, two cases showed metastatic disease. In one case primary site was located in middle phalanx of left hand and metastatis to lungs, vertebrae and brain. In another case primary was in iliac bone with secondaries in vertebra. Approximately 20-30% patients have evidence of metastasis at diagnosis involving lung and bones (13).

Two cases of Hepatoblastoma diagnosed on FNAC were confirmed on HPE.

One case of Ependymoma diagnosed on squash cytology (Fig.8) was confirmed on HPE (Fig.9).

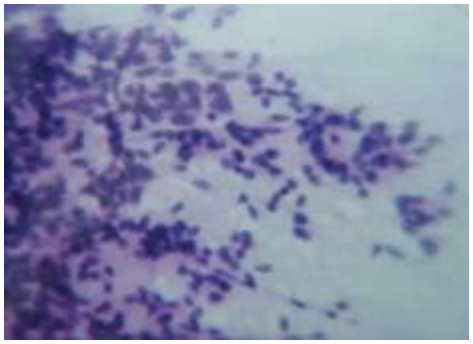

Fig-8; Ependymoma; Squash cytology

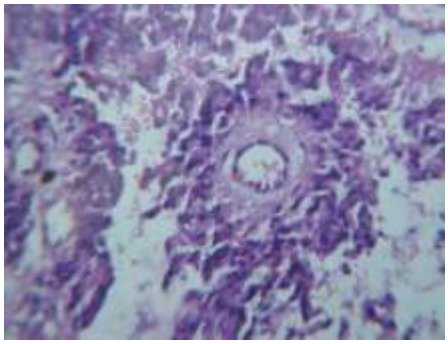

Fig-9; Ependymoma; HPE

One more case of Medulloblastoma diagnosed on squash cytology was also confirmed on HPE. Only one case of Pancreatoblastoma was diagnosed on HPE.

VI. Summary And Conclusion

A study of Blue round cell tumors was carried out over two year period with regards to age and sex prevalence, clinical presentation, cytological findings, macroscopic appearance, histologic typing and immunohistochemistry in a few. Literature was collected on various aspects of childhood SBRCT and the following conclusions were drawn.

Total number of tumors studied was 56. Special emphasis was given on study of correlating cytological diagnosis with histopathological examination and IHC in few doubtful cases.

Lymphomas and nephroblastomas contributed the majority of SBRCT followed by neuroblastomas, rhabdomyosarcomas and Ewing's sarcoma. Hepatoblastomas were two. medulloblastoma, Ependymoma and pancreatoblastoma being only one each..

Most of the nephroblastomas, neuroblastomas and hepatoblastomas appeared in the age group of less than 5 years whereas majority of other small blue round cell tumors appeared after the age of 5 years.

Out of the 41 cases for which cytology was done 7 cases were unfit for surgery. Hense only for 34 cases both FNAC and HPE are available. Of 34 cases 30 (88%) cases correlated with histopathological diagnosis. In 7 cases which were unfit for surgery, cytological diagnosis helped the oncologist to treat the patients.

Immunohistochemistry was useful in typing the tumors in few cases where conclusion by histopathological examination alone was not possible .

In case of neuroblastomas and rhabdomyosarcomas grading was given and in case of wilm's tumor prognostic factors helped in further treatment and follow up of patients.

Since many paediatric Small blue round cell tumors lack differentiation and mimic other small cell tumors, routine methods are often insufficient to resolve problematic histology. Our study provided accurate histological categorisation of resected or biopsied tumors and grading helped in accurate treatment and in assessing prognosis.

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