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Original Research Article

Efficacy of cytology in small round cell tumours

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Abstract

Background: Malignant small round cell tumours are characterized by small, round, relatively undifferentiated cells sharing similar histology when visualized under light microscope. These typically encompass Ewing's sarcoma, peripheral neuroectodermal tumour (PNET), rhabdomyosarcoma, synovial sarcoma, non-Hodgkin's lymphoma, retinoblastoma, neuroblastoma, hepatoblastoma, and nephroblastoma commonly known as Wilms' tumour. Fine needle aspiration cytology (FNAC) has emerged as a vital diagnostic tool for these types of tumours.

Materials and Methods: This was a retrospective study of all diagnosed cases of pediatric small round cell tumours in Maulana Azad Medical College and Associated LNH Hospital, New Delhi between the period of 2016-2020. 30 cases of pediatric small round cell tumour were studied. Data was analysed using SPSS version 25 and MS Excel. Qualitative data was expressed as frequencies, percentages and proportions. Sensitivity and Specificity of FNAC in categorization of small round cell tumours was calculated.

Results: In 22 out of 30 cases, FNAC could correctly establish the correct nature of lesion with an overall diagnostic accuracy of 73.3%. Complete concordance (diagnosis of SRCT with further categorization) could be done in 22/30 (73.3%) cases, whereas discordance in subtyping was seen in 8/30 (26.6%) cases.

Conclusion: FNAC could correctly establish the correct nature of lesion with an overall diagnostic accuracy of 73.3%. Adequate clinical history and classical presentations assisted significantly in arriving at the final diagnosis. Clinical and radiological correlation aided with ancillary techniques increases the diagnostic accuracy of FNAC in Malignant small round cell tumour.

Keywords: Small round cell tumour, Fine needle aspiration cytology, Ewing's sarcoma, rhabdomyosarcoma, non-Hodgkin's lymphoma, Wilms' tumour.

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1. Introduction

Small round cell tumours (SRCTs) represent a diverse group of neoplasms that predominantly occur in children and adolescents.[1] These tumours are characterized by small, round and relatively undifferentiated cells that display similar histological features under light microscopy.[2] Common types include Ewing's sarcoma,[3,4] peripheral neuroectodermal tumours (PNET), rhabdomyosarcoma,[5] synovial sarcoma,[6] non-Hodgkin's lymphoma, retinoblastoma,[7] neuroblastoma, nephroblastoma (Wilms' tumour) and hepatoblastoma.

The challenge of accurately diagnosing SRCTs arises from their primitive and undifferentiated characteristics. While tumours that are well-differentiated are typically easier to classify, the lack of distinctive morphological features in poorly differentiated cases complicates the diagnostic process. Fine needle aspiration cytology (FNAC) has proven to be a crucial method for diagnosing these tumours, as it can yield sufficient viable cells for further analysis using techniques such as immunocytochemistry, flow cytometry, and electron microscopy.[8,9] Recent research has shown that FNAC can achieve a definitive diagnosis in approximately 57% to 68% of cases.[10-12]

This study aims to assess the effectiveness of FNAC in sub-classifying SRCTs and to outline the cytomorphological characteristics associated with different tumour types. The objectives include categorizing SRCTs based on clinical, cytological, and immunocytochemical data, and correlating these findings with histopathology, which is regarded as gold standard for the diagnosis.

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2. Materials and Methods

This was a retrospective study of all diagnosed cases of paediatric small round cell tumours in Maulana Azad Medical College and Associated LNH Hospital, New Delhi between the period of 2016-2020. The pathology reports were retrieved, and information about the age, sex, anatomical site, clinical and histological diagnosis were extracted. The paraffin blocks of all available cases of SRCT which met inclusion criteria diagnosed in the study period were retrieved. Fresh sections were prepared and stained with hematoxylin and eosin (H and E) where ever needed. Detailed cyto-morphological evaluation was done and correlated with histopathology along with immunohistochemical findings. 30 cases of paediatric small round cell tumour were studied.

Following methods of sample acquisition were included:

1. FNAC smears
2. Cell block (CB) preparation
3. Small round cell tumour biopsy/specimens

2.1. FNAC technique

A written and informed consent/assent was taken from all patients, patient's parents or guardians and procedure was explained to them. Demographic details and relevant clinical history (including past and family history) were recorded. General physical, local and systemic examination was conducted. Details of palpable soft tissue mass if present and evidence of metastasis if present was noted. Under strict aseptic conditions, FNAC was performed without radiological guidance with a 22 to 23 gauge needle attached to a 10ml disposable plastic syringe. Needle was introduced into the swelling and gently manipulated into various directions within the swelling while maintaining negative pressure and by giving multiple passes if needed. In cases of cystic lesion the smears were prepared from cyst fluid after cyto-centrifugation. For cell block preparation blood mixed aspirate was allowed to clot and subsequently fixed in formalin and was processed further as routine histopathological sections.

2.2. Processing of the sample

The aspirated material was smeared on to clean 6-7 glass slides. One smear was wet fixed and PAP stain was applied. Two smears were air dried and fixed in methanol for 10 minutes at room temperature and then stained with Giemsa stain. Rest of the smears were wrapped in three layers of aluminium foil and preserved at 0° Celsius in refrigerator for immunocytochemistry (ICC).

The CB were processed as routine paraffin embedded section. CBs were fixed in 10% buffered formalin and then processed in histokinette, embedded in paraffin block. Sections of 4 micrometers thin were cut using microtome. Staining was done as routine hematoxylin and eosin (H&E) stain. The sections were studied in detail for morphological typing.

Immunocytochemistry (ICC) was applied on cell block/aspirate smears. Tumours were then subcategorized based on cytomorphological features and their immunocytochemistry (ICC) findings.

2.3. Processing of tissues

All the resected specimens were fixed in 10% buffered formalin. Complete gross examination was done with special emphasis on, type of surgical specimen, size of the tumour, Gross appearance of the tumour, site of the tumour, number of lymph nodes isolated. After fixation the sample was processed in histokinette for 15.5 hours. Paraffin blocks were prepared and multiple 5 micrometer sections were cut using a rotary microtome. Sections were mounted on clean slides for H&E staining.

2.4. Immunocyto & histochemical staining

Primary antibody used-CK, S-100, SMA, EMA, CD34, Vimentin. DAB (diaminobenzidine)-used as a substrate for peroxidase (used as an enzyme label). It forms a stable brown end product at the site of the target antigen (Ag). Citrate buffer-freshly prepared each time and used for antigen retrieval. PBS (phosphate buffered saline) buffer is used and Harris hematoxylin-used as a counterstain.

2.5. Method

All steps were carried out in a moist and humid chamber and care was taken to ensure that the sections remained moist throughout the procedure. For FNAC smears, Air-dried smears were fixed in a mixture of cold acetone and methanol (1:1) at 20°C for 5 minutes, for Paraffin sections. The staining area on the section slides was marked. The four micron sections were deparaffinised by putting them on a hot plate and by dipping them in xylene. They were then hydrated with graded ethanol followed by being brought to water. To block the endogenous peroxide activity, slides were placed in 3% H2O2 in methanol (H2O2 block) for 30 minutes at room temperature. The slides were given 3 washes with PBS buffer. A coplin jar was filled with 10mM citrate buffer (pH-6) and covered with a lid. The slides were then put in the prewarmed buffer, covered with a cling film and heated in microwave for 40 minute which allowed unmasking of the epitope. The slides along with the buffer were allowed to cool down, then washed thrice with PBS buffer, and excess buffer was wiped off using a filter paper strip. The tissue was incubated with primary antibody overnight at 4°C in a moist dark chamber to prevent drying. Three washes with PBS buffer were given; excess buffer was wiped by filter paper strip. Secondary antibody (biomethylated goat antipolyvalent antibody) was placed on the section, incubated in moist chamber for 30 minutes. Three PBS buffer washes were given; excess buffer was wiped by filter paper strip. Tertiary antibody (peroxidase labelled streptavidin-peroxidase

complex) was placed on the section, incubated in moist chamber for 30 minutes. Three washes with PBS buffer were given; excess buffer was wiped by filter paper strip. DAB was applied on the slides and the reaction monitored under the microscope. Once the colour developed, the sections were put in distilled water to stop the reaction. The slides were counterstained with Harris haematoxylin for 2 minutes. They were dehydrated in graded alcohol solutions. They were then passed through xylene which acted as a clearing agent. Then finally the slides were mounted with DPX. Cyto-histological (CB and/biopsy) correlation was performed for final diagnosis.

2.6. Statistical analysis

Data was recorded in an MS Excel spreadsheet and analysed using SPSS version 25. Qualitative data were presented as frequencies, percentages, and proportions. The diagnostic accuracy of FNAC in classifying small round cell tumours was assessed, along with the calculation of its sensitivity and specificity.

3. Results

A total of 36,327 FNACs were performed in pathology department of pathology, Maulana Azad Medical College during the study period (January 2016 to December 2020). Out of these, 158 cases were reported as small round cell tumours. However, subsequent his to pathological correlation was available only in 30 cases, which were included in thestudy.

3.1. Distribution of cases

All SRCT cases were divided into 4 categories: Neurogenic origin, Mesenchymal origin, Hematolymphoid origin and malignant small round cell tumours of uncertain type. All 30 cases were distributed category wise. Out of 30 cases: 10 cases were of neurogenic origin, 4 cases mesenchymal origin, 15 cases hematolymphoid origin and 2 cases were of malignant small round cell tumour of uncertain type.

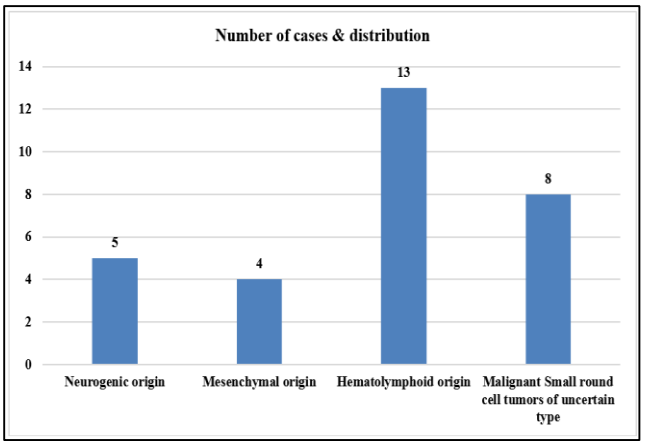

Figure 1: Number of cases & distribution

3.2. Age distribution

The age ranged from 6 months to 18 years with mean being 11.08 years and median being 12 years. Majority of patients (11/30) lied in age group of 15 to 18 years.

Table 1: Age-category wise distribution

Age group (years)	Neurogenic origin	Mesenchymal origin	Hematolymphoid origin	Malignant small round cell tumors of uncertain type	Total
≤ 4 year	1	1	2	0	4
5 – 9 years	3	0	1	3	7
10 – 14 years	1	1	4	2	8
≥15 years	0	2	6	3	11
Total	5	4	13	8	30

Table 2: Gender and category wise distribution

Gender	Neurogenic origin	Mesenchymal origin	Hematolymphoid origin	Malignant small round cell tumours of uncertain type	Total
Male	3	3	3	7	16
Female	2	1	10	1	14
Total	5	4	13	8	30

3.3. Gender distribution

Out of 30 cases, 16 were male and 14 were female, with a ratio of 1.14:1.

3.4. Laterality of involvement

In most cases (21/30) lesions were unilateral, while 6/30 cases had bilateral involvement.

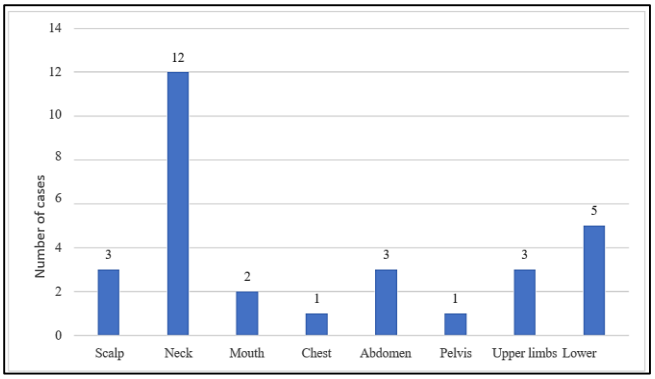

Figure 2: Site distribution

3.5. Focus of involvement

In most cases (23/30) lesions had a single focus, while seven cases had multiple foci. (≥2 foci)

3.6. Symptoms at presentation

In majority of cases (19/30) lesions had no pain, while eleven cases had pain during presentation.

Table 3: Symptoms at presentation

Skin involvement	Pigmentation	No. of Cases	Percentage
Nodule, intact skin	Present	2	6.7%
	Absent	26	86.7%
Nodule, ulcerated skin		2	6.7%

3.7. Sub-category wise distribution

All cases were distributed into different categories on the basis of cell of origin.

Table 4: Subcategory wise distribution according to outcome on cytology

S. No.	Categories	Subcategory	No. of cases	Percentage
1.	Neurogenicorigin	Ewing's sarcoma	3	10.0%
		Neuroblastoma	1	3.3%
		Wilm's tumor	1	3.3%
2.	Mesenchymal origin	Osteosarcoma	1	3.3%
		Rhabdomyosarcoma	3	10.0%
3.	Hematolymphoid origin	Hodgkin's lymphoma	5	16.7%
		Non-Hodgkin's lymphoma	8	26.7%
4.	Malignant small roundcell tumorsof uncertain type	Malignant SRCT	8	26.6%

Table 5: Distribution of cases according to outcome on histopathology

S. No.	Categories	Subcategory	No. of cases	Percentage
1.	Neurogenic origin	Ewing's sarcoma	6	20.0%
		Neuroblastoma	2	6.6%
		Wilm's tumor	1	3.3%
2.	Mesenchyma l origin	Osteosarcoma	1	3.3%
		Rhabdomyosarcoma	3	10.0%
3.	Hematolymp hoid origin	Hodgkin's lymphoma	5	16.7%
		Non-Hodgkin's lymphoma	10	33.3%
4.	Malignant small round cell tumors of uncertain type	Malignant SRCT	2	6.6%

On the basis of cytology 4 cases were diagnosed as SRCT (neurogenic origin), Ewing's sarcoma was the commonest (3/5 cases). Among the second category SRCT (mesenchymal origin), Rhabdomyosarcoma was commonest (3/4 cases). The maximum number of SRCT cases (13/30 cases) belonged to SRCT (hematolymphoid origin) and in this category Non- Hodgkin's lymphoma was the commonest (8/13 cases). Further sub categorization of NHL was not possible on cytology. The fourth category, Malignant SRCT of uncertain origin accounted for 8/30 cases which was the second highest cases after hematolymphoid malignancy.

Out of 30 cases included in the study, on histopathology 9 cases were given as Malignant SRCT(Neurogenic origin), but on cytopathology only 5 cases were categorized as Malignant SRCT(Neurogenic origin), however in 4 cases diagnosis of Malignant SRCT was given and no further sub categorization was possible.

On histopathology 4 cases were reported as Malignant SRCT (Mesenchymal origin) and on cytopathology correct sub categorization was possible in all 4 cases. Among the Malignant SRCT (Hematolymphoid origin), category 15 cases were diagnosed on histopathology but on cytopathology only 13 cases were sub categorized as Malignant SRCT (Hematolymphoid origin), however 2 cases were diagnosed as Malignant SRCT of uncertain type and no further sub categorization was possible. 4 cases were categorized as Malignant SRCT of uncertain type on both histopathology and cytopathology.

Among SRCT(neurogenic origin), 3/6 cases of Ewing's sarcoma diagnosed on cytology were concordant with histopathology however in 3/6 cases of Ewing's sarcoma were discordant on further subtyping and only broad sub categorization of Malignant SRCT was possible. Similarly, 1/2 cases of neuroblastoma was concordant while 1/2 case was discordanton sub typing. Single case of wilm's tumour diagnosed on cytology was concordant with histopathology.

Among SRCT (mesenchymal origin), all 3 cases of RMS and 1 case of osteosarcoma diagnosed on cytopathology were concordant with histopathology.

Among SRCT (hematolymphoid origin), all 5 cases of Hodgkin's lymphoma were concordantwith histopathology, while in NHL concordance was less as compared to Hodgkin's lymphoma and 8/10 cases were concordant with histopathology, while 2 cases were discordant on subtyping.

4. Discussion

Fine Needle Aspiration Cytology FNAC) is a useful tool for the diagnosis of Small Round Cell Tumours (SRCT) in paediatric population. There are studies across the globe which have compared it with histopathology and they have drawn a good correlation between the two. Many studies have used other ancillary techniques like Immunohistochemistry, flow cytometry and molecular studies for comparing its diagnostic accuracy. This is a retrospective study, where data of last 5 years of small round cell tumour cases diagnosed in the department of Pathology at Maulana Azad Medical College, Delhi were analysed. In a similar study in India by Maheshwari et al. 588 cases over a period of ten years were studied.[13] This study was conducted at JN Medical College, AMU, Aligarh, is a retrospective as well as prospective study and included patients younger than 15 years of age. Clinical, cytopathological and histopathological correlation was performed in all cases. Out of the 207 malignant cases, Lymphomas were the most common tumours (25.1% cases) and non Hodgkin's lymphoma (14.5% cases) were more common than Hodgkin's lymphoma (10.6% cases) which is quite similar to our study. In our study SRCT of hematolymphoid origin was the commonest category (50% cases). NHL (26.7% cases) was commoner than Hodgkin's lymphoma(16.7% cases) Male to female ratio was 2:1, whereas in our study it was close to 1.1:1 with a slight male preponderance. In another study performed by McGahey et al 39 cases of SRCT were studied in a prospective manner over a period of 4 years in 2 hospitals in USA.[8] The most common tumour in this study was lymphomas (54%). Ewing's sarcoma (18%) was the next common tumour. In our study after Lymphomas the next common category was Malignant SRCT of uncertain type (26.6% cases) and SRCT(neurogenic origin) was the third most common type of tumour among which Ewing's sarcoma was commonest and accounted for 10% of the cases. Halliday et al also studied 20 cases of SRCT and they evaluated the utility of MIC-2 antibody which is considered a sensitive and specific marker of ES and Peripheral Neuro Ectodermal Tumour (PNET).[14] Twenty cases of small blue cell neoplasms were obtained by FNAC and MIC-2 antibody was applied in retrospect to formalin-fixed cell block material and de-stained alcohol-fixed and air-dried cytologic preparations.

The cases were considered positive when majority of the cells showed cytoplasmic staining and it was observed in all seven cases of ES & PNET. Staining could be observed on the destained air-dried smears (three cases), fixed smears (two cases), or the cell block material (two cases) whereas, none of the other 13 small blue cell neoplasms showed positive staining. This study highlighted the diagnostic utility of MIC-2 antibody in the differentiation of ES/PNET from other subgroups of SRCT. Leon et al studied 3 cases with flow-cytometry immune-phenotyping (FCI) demonstrated that and ES/PNET in FNAC can be efficiently and rapidly diagnosed by combining cytologic examination with FCI using a panel including CD45, CD16/56, and CD99.[15]

Diagnostic correlation Cytopathology and Histopathology was observed in 218 out of 226 cases by Maheshwari et al giving an overall diagnostic accuracy of 96.4%.40 The sensitivity and specificity of FNAC were found to be 95.8 and 97.6% respectively, in the same study. In our study Cytopathology and Histopathology Concordance

was seen in 22/30 cases (73.3%), whereas 8 cases also were discordant on subtyping. McGahey et al concluded that in pediatric patients, FNAC specimens should be evaluated by a skilled cytologist at the time of procurement and ancillary studies selected at that time. Using additional diagnostic techniques, FNAC of SRCT is a safe and accurate technique for staging, documenting recurrence, or initial diagnosis in pediatric patients. Gangopadhyay et studied 88 cases of non-hematological origin in pediatric population out of SRCT comprised of 44% cases.[16] Definite diagnosis was possible based on the cytomorphology in 79.5% cases, while in 20.5% of cases only a broad cytological classification could be offered. Asim et al studied 35 cases of SRCT and established a correlation of FNAC keeping Histopathology as the Gold standard for the diagnosis.[17] In this study, the most common malignant small round cell tumour (SRCT) on histopathology was Wilms tumour (10 cases) followed by Non Hodgkin lymphoma (9 cases). FNAC results were correlated with the histological findings and the diagnostic accuracy of SRCT was found to be 98%. The sensitivity and specificity of FNAC in diagnosis of SRCT was 97% and 100% respectively. FNAC was found to be a very useful technique in the initial evaluation of any palpable lesion of childhood. Although the small round cell tumours appear cytologically similar, in the hands of experienced cytopathologists, the subtle morphological features can help towards the final diagnosis. It was further observed that clinical and radiological findings along with the judicious use of ancillary techniques can further increase the diagnostic accuracy.

5. Limitations of the Study

1. The nature of the study is retrospective.
2. The sample size of the study is 30. A larger sample size would have resulted in increase of the statistical power and generalizability of the findings.

6. Conclusion

The present study was conducted in the department of Pathology of Maulana Azad Medical College and Associated hospitals. A total of 158 patients diagnosed as small round cell tumour on cytology were followed up out of which histopathology specimen was received for 30 patients.

Following conclusions were drawn based on the results and observations:-

1. The patients ranged in age widely from 6 months to 18 years, maximum patients (36.66%) were in the age group of 15-18 years.
2. There was a male preponderance with M:F ratio of 1.14:1.
3. Head and neck was the most common site of involvement, followed by extremities and trunk; most lesions were present over the neck.
4. The most common presentation of Small round cell tumours was small and painless nodular swelling, majority <6 cm with few cases showing pigmentation and surface ulceration.
5. In 22 out of 30 cases, FNAC could correctly establish the correct nature of lesion with an overall diagnostic accuracy of 73.3%.
6. Complete concordance (diagnosis of SRCT with further categorization) could be done in 22/30 (73.3%) cases, whereas discordance in subtyping was seen in 8/30 (26.6%) cases.
7. Adequate clinical history and classical presentations assisted significantly in arriving at the final diagnosis.
8. Major reason for inability to further subcategorize the Small round cell tumours was overlapping cytomorphological features.
9. The reason for discordance on subtyping in 8 cases were because of unusual site of lesions, morphologic overlapping features and also sampling error/limited sampling especially from smaller, deep seated and difficult to approach lesions.
10. Clinical and radiological correlation aided with ancillary techniques increases the diagnostic accuracy of FNAC in Malignant SRCT.

7. Source of Funding

None.

8. Conflict of Interest

None.

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