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Pediatric Fluid Cytology: A 2-year experience of a tertiary care pediatric center of North India

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ABSTRACT

Background: Body fluid analysis containing exfoliated cells in effusion may reveal information about neoplastic and non-neoplastic etiology. Most important indication of fluid cytology is to look for malignant cells. CSF examination is another investigation performed routinely in all tertiary care centers in addition to other body fluids examined. **Aims and Objective:** The aim of the study was to examine the body fluids for cytological examination in diagnosis, prognostic and therapeutic tool for management of pediatric cases. **Materials and Methods:** Retrospective data analysis of 170 fluid cytology cases taken from departmental data archive of pediatric cases (0-18 years) from Jan 2018-Dec 2019 was tabulated observation was done by 2independent pathologists. The smears were stained with Romanowsky stains and specialized stains of AFB and Rhodamine auramine, MPO, PAS and PAP as per requirement. **Results:** There were higher number of male (n = 99, 58.23%) compared to female (n = 71, 41.76%). The commonest fluid cytology received was the CSF (n = 150, 88.23%) with 77 samples presenting with significantly high TLC for age (using standard age analysis parameters). The diagnosis was grouped into the Neoplastic and Non neoplastic category. The non-neoplastic category was further divided into infective where most common was viral pleocytosis (n = 50, 48 Viral lymphocytic pluecytosis, 1case rubella positive CSF, 1 Primary HLH with viral infection) followed by TB(n = 10, 6 pleural, 2 CSF, 2 Peritoneal), bacterial (n = 07, microbiology culture was done) and TORCH, Rubella with plasmacytosis (n = 01). Another non-neoplastic category was of inherited disease where single case of Griscelli's syndrome was diagnosed showing hemophagocytosis on CSF examination. Neoplastic Cytology was seen in 07 cases where all the cases were hematolymphoid malignancies. **Conclusion:** This study concludes that fluid cytology is a useful diagnosis, prognostic, therapeutic tool in diagnosis and management of pediatric cases.

Key words: Audit; Cytology; Fluid; Pediatrics

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INTRODUCTION

Cytological evaluation of body fluids has always been a basic yet essential investigative practice in the field of cytopathology. Cytological evaluation of fluids is a relatively simple, quick, inexpensive and minimally invasive technique with high accuracy and low incidence of false negative diagnosis. Common fluids examined are pleural, peritoneal, pericardial, cerebrospinal fluid (CSF) and synovial fluid. These fluids undergo abnormal and disproportionate qualitative and quantitative changes during a disease process.[1,2] The overall cytological evaluation with clinical, radiological, and physical examination is an aid to primary provisional diagnosis.

Fluid cytology is commonly used to diagnose a spectrum of both non Neoplastic and Neoplastic (benign and malignant) diseases. It is of diagnostic, prognostic and therapeutic importance with a high sensitivity and specificity.[2] This could be due to the fact that exfoliative cytology covers a

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larger area of the organ which is exposed to the fluid in which the cells exfoliate rather than a site-specific biopsy. Our set up is a pediatric set up hence we get a number of fluid samples like CSF, pleural fluid and peritoneal fluid and sometimes pericardial and synovial fluids for assessment. This study is an attempt to audit our experience of pediatric cases for fluid cytology over a period of two years.

MATERIALS AND METHODS

This is a retrospective observational descriptive study which was conducted in the Department of Pathology, Super Speciality Paediatric Hospital and Post Graduate Teaching Institute, Noida where archived data from Jan 2018-Dec 2019 was analyzed and a total of 170 cases were compiled and tabulated. Only new samples were included in the study and the common fluids received were CSF, pleural and ascitic fluid. Cases with repeat CSF for therapeutic monitoring or old diagnosed cases were excluded. All the cases belonged to the institute's pediatric department. Complete patient history, clinical details and other relevant investigations of the patient were retrieved from cytopathology form filled at the time of submission. All fluids were subjected to Cytospin [Thermofisher] preparation as well as centrifuge preparation for extra smears for special stains. Slides were stained by Romanowsky Stains (Leishman, Giemsa) and the smears were processed for specialized stains of Ziehl Neelsen staining for Acid fast bacilli and Rhodamine auramine, Myeloperoxidase, Periodic acid Schiff and Papanicolaou stains as per case requirement. The slides were evaluated on light microscopy for Cellularity, Predominant cell type, Size, Architecture, Nuclear and Cytoplasmic features, Chromatin, Degree of inflammation, Reactive changes and other background features. The diagnosis for the fluids has been made independently by two independent pathologists thereby limiting chances of observer bias. The smears were processed for specialized stains of Ziehl Neelsen staining for Acid fast bacilli and Rhodamine auramine, Myeloperoxidase, Periodic acid Schiff and Papanicolaou stains as per case requirement.

RESULTS

On analyzing our data of 170pediatric cases [up to 18yrs], we found 120 cases between the agesof 0-10years with males>females (Table 1). The common fluid for cytology received by us was the CSF (n=150/170) while rest 20 were other body fluids. Out of the total cases, 77 cases were present with significantly higher TLC for the reference age (using standard age analysis parameters). On analyzing 150 CSF cytology cases, the lesion was grouped into the Non neoplastic category which was further divided as per their etiology, where most common was viral pleocytosis (48 Viral lymphocytic pleocytosis, 1case rubella positive CSF, 1 Primary HLH with viral infection) followed by TB(n=10, 6 pleural, 2 CSF (Figure 1 showing AFB positive), 2 Peritoneal), bacterial (07) and 01 case with plasmacytosis in CSF (Figure 2) which was diagnosed as of TORCH (Rubella Positive) and 1 diagnosed case of Griscellis syndrome on follow up showed hemophagocytosis (Figure 3) on CSF examination. On CSF cytology smears for malignant cells 07 cases ALL (Figure 4, lymphoblast PAS positive), APML (Figure 5) and T-NHL in pleural fluid (Figure 6), were malignant (total cases n=7/170).

Table 1: Demographic data (better to mention % for each of these cases and total frequency and percentage as well)

0-10 years (120 cases)		11-18 years (50 cases)	
Males	**Females**	**Males**	**Females**
82(48.23%)	38(22.35%)	17(10%)	33(19.41%)

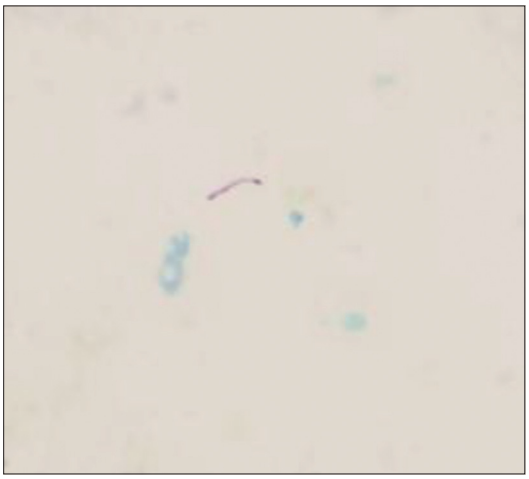

Figure 1: Zhiel-Neelson (stain) shows positive AFB in CSF (1000X)

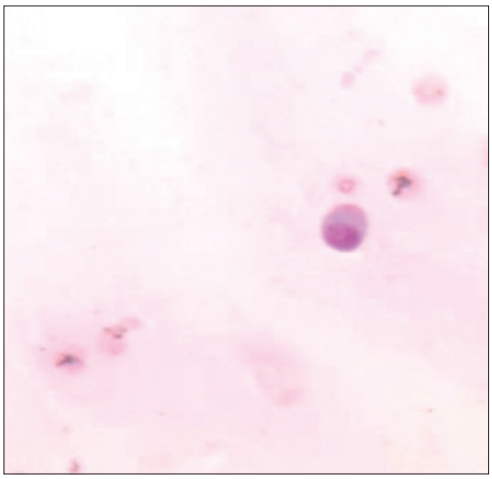

Figure 2: An occasional plasma cell seen in CSF(Leishman Stain, 400X)

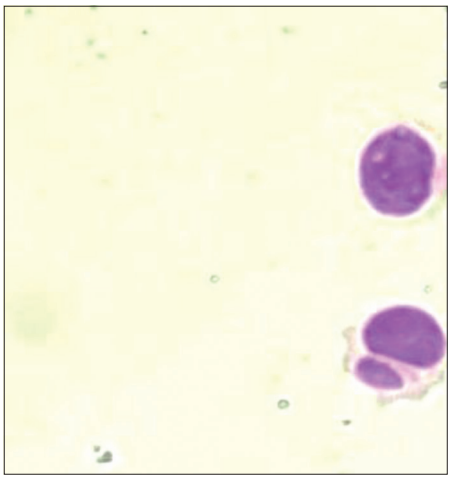

Figure 3: Hemophagocytosis in monocyte in CSF(Leishman Stain, 1000X)

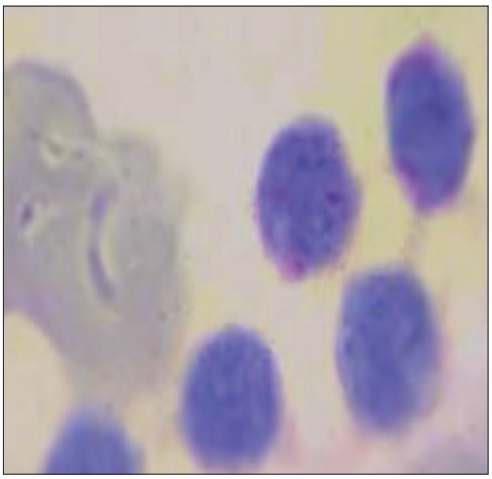

Figure 4: PAS positive blast cells in CSF fluid in a case of ALL(PAS Stain, 1000X

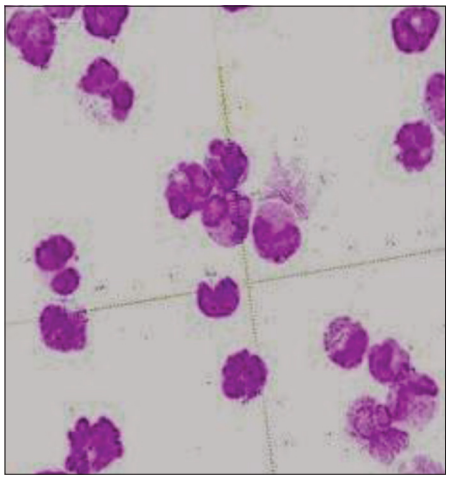

Figure 5: APML spreading in CSF (Leishman stain, 400X)

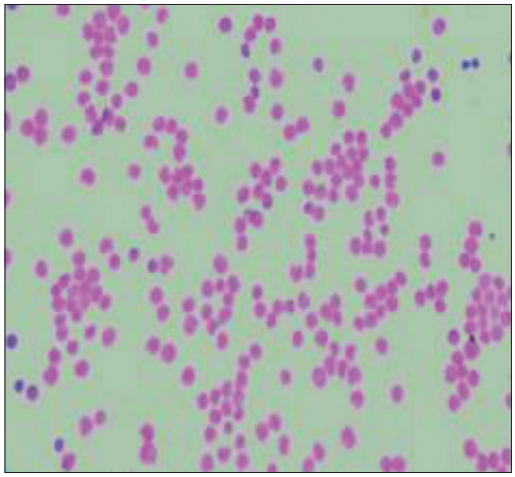

Figure 6: Lymphoma cells in pleural fluid. (T-NHL cell, Leishman stain, 400X)

The demographic data (Table 1), Differential diagnosis of CSF Cytology (Table 2) and Differential diagnosis of other fluids (Table 3) and the Types of Body fluid (Table 4) have been explained below.

DISCUSSION

For decades, body fluid analysis has played an important role as a diagnostic aid in establishing a definitive diagnosis, predicting prognosis and planning or monitoring therapy. It has gained increased acceptance in clinical practice today, since it is relatively simple, safe and inexpensive procedure.[3,4] The number of samples received in pathology laboratory is increasing and the clinicians use the effusion cytology report to diagnose and treat the underlying cause. Our department being a part of a pediatric center regularly receives samples for fluid analysis from various departments with cases ranging from leukemia at the time of diagnosis and on follow up with lymphoma and other malignancy, PNET, Rhabdomyosarcoma, Ewing sarcoma, NK cell leukemia to suspected infections (parasitic, bacterial, viral and fungal). Cytological evaluation of body fluids is diagnostically challenging. Cytomorphological examination of pathological body fluid is a well-accepted method to categorize them as Neoplastic (benign or malignant) or non-neoplastic. By far, the recognition of malignant cells is the most important goal of fluid cytology and this is often used as a first line of investigation to detect and typify metastatic disease based on subtle morphological features.[5-8] The presence of malignant cells in body fluids indicates spread of disease beyond the organ of origin and this is important both therapeutically as well as prognostically.[7] In this study we have attempted to document our experiences with pediatric fluid analysis and small sample size is one limitation of this study.

In the present study, a total of 170 cases of fluid specimens were studied. Most common fluid received was CSF

Table 2: Differential diagnosis of CSF

S.No.	Non-Neoplastic cytology		Neoplastic cytology			
1	TB Lymphocytic pleocytosis	02	Benign	cases	Malignant	cases
2	Viral Lymphocytic pleocytosis with activated lymphocytes	48	CSF Negative for malignant cells	92	ALL	05
3	TORCH(Rubella) Serology positive	01			APML	01
4	Primary HLH(Griscelli syndrome-type II) Activated monocytes	01				

Table 3: Differential diagnosis of other fluids

S.No.	Non-Neoplastic		Neoplastic			
1	Diagnosis	cases	Benign	cases	Malignant	cases
2	Pleural Fluid (Empyema)	03	-	-	T-NHL (Pleural Fluid)	01
3	Pleural Fluid (TB)	06				
4	Peritoneal Fluid (Lymphocytic effusion ?TB)	02	Negative for malignant cells	03		
5	Pericardial (suppurative)	02				
6	Synovial fluid (septic arthritis)	02				
7	Synovial fluid PVNS	01				

*Benign/non neoplastic-reported as negative for malignant cells
**malignant-reported as positive for malignant cells

Table 4: Types of fluid (mention percentage and total frequency and percentage)

CSF	Other fluids
150 (88.23%)	Pleural Fluid-10(5.88%) Ascitic Fluid-5(2.94%) Pericardial Fluid-2(1.17%) Synovial fluid-3(1.76%)

(88.23%), followed by pleural (5.88%). This is different from the findings in few other studies[8-10] who had pleural fluid as the most commonly received sample. This difference could also be due to the fact that our patients are purely pediatric oncology cases which have more of CNS involvement than pleural or peritoneal involvement in common.

Samples from the male patients were more as compared to the female in the age groups of 0-10 years while the reverse was observed for the age group 11-18 years (Table.1). This was in concordance with most of the studies by other authors.[11-15] A study on purely pediatric sample has not been conducted in detail to the best of our knowledge hence marked difference in age of presentation seen from other studies.

The interpretation of malignancy is difficult in body fluids. This may be due to a smaller number of malignant cells present in the fluid which may go unrecognized on cytological examination leading to false-negative diagnosis. Also, reactive mesothelial cells seen in body fluids except CSF where arachnoid cells might confuse an unexperienced eye, may mimic malignant cells in conventional cytological smears, largely because reactive mesothelial cells show nuclear enlargement and hyperchromasia, with or without presence of prominent nucleoli and they may be arranged in rosettes, pseudoacini or acini, resulting in a false- positive diagnosis.[1,12,16,17] In our center most of the malignant cases diagnosed were straight forward however we did face problems in diagnosing case of primary HLH on follow up for persistent hemophagocytosis due to the extensive background mixed pleocytosis. Another confusing finding in a follow up case of leukemia with persistent mixed pleocytosis was large bizarre activated monocytes with fragmentation of nucleus which if not looked at carefully will mimic a hemophagocytosis. Viral lymphocytes which are activated can mimic blasts and confuse an untrained eye.

In the present study, out of total 170 fluid cytology cases with 7 (6 CSF, and 1 pleural) cases were, malignant and the remaining were non neoplastic. These findings were similar to some studies conducted on adult as well as pediatric population mixed.[12,18,19] Out of 7 malignant effusions, 6[acute leukemia] were CSF and 1 [T-NHL] was pleural. In the non-neoplastic effusions 1 Griscelli's Syndrome case was on follow-up and CNS symptoms showed CSF with hemophagocytosis, 1 case was diagnosed as PVNS on synovial fluid examination, 10 cases were of tuberculosis, 6 were from pleural fluid 2 were from CSF and 2 were from ascitic fluid. These cases were confirmed on gene expert and by fluid ADA levels.

The bacterial infection was confirmed on grams staining and culture. The common fluids involved by the infection were pericardial effusion, Synovial fluid, pleural CSF etc. PAS, MPO, PAP, AFB, Gram's stains were used to differentiate blasts, isolate organisms or to confirm presence of malignancy where ever required.

CONCLUSION

Cytological examination of fluid is a simple cost effective yet important diagnostic modality. It is useful to diagnose, determine disease progression and to pin point etiology of effusion. A wide spectrum of pediatric diseases can be diagnosed on fluid cytology and proper management can be meted out to the patient. With the use of ancillary investigation immunocytochemistry, Special Stain, flow cytometry, it can give very high accuracy in results especially in pediatric malignancy Leukemia/lymphoma other round cell tumors involving CNS and other body fluids. Hence we conclude that fluid cytology should be included as a routine practice in peripheral as well as tertiary health care centers and it is of great value in pediatric set up.

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SS- Concept, manuscript formulation data analysis; **DN**- Editing and review; **NT**- Editing, manuscript formulation

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