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CEREBROSPINAL FLUID CYTOLOGY IN CANCER PATIENTS: AN INSTITUTIONAL STUDY WITH 5394 CASES

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ABSTRACT

Objectives: Cytological examination of cerebrospinal fluid (CSF) is a routine procedure in the management of patients with malignancies that frequently spread via leptomeninges. Interpretation, understanding and cytological characteristics of these lesions are required. Detection of malignant cells in cerebrospinal fluid in cytology is the diagnostic gold standard for leptomeningeal carcinomatosis (LC).

Material and Method: Retrospective analysis of 5394 cases of CSF was done over 5 year's duration at cancer hospital. Smears were stained with papanicolaou stain and examined. Data were analyzed with clinical detailed.

Results: Patient's age ranged from 1 to 70 years. Out of 5394, 273 cases were positive for malignant cells, and 4 cases had infectious etiology. Acute lymphoblastic leukemia was the commonest malignancy infiltrating CSF in both, followed by retinoblastoma, nonhodgkins lymphoma in pediatric and nonhodgkins lymphoma and metastatic carcinoma in adults. Other rare tumor infiltrating CSF found in our study were myeloid leukemia, medulloblastoma, peripheral neuroectodermal tumor, and space occupying lesion of brain, where primary could not found. Of four infectious cases, one case of Cryptococcus meningitis was found.

Conclusion: Correct identification of LC is important as it has therapeutic and prognostic implications. Thus, cytological examinations of the CSF played a decisive role in the diagnosis of LC.

Key Words: Cerebrospinal fluid, Cytology, Meningitis, Cancer, CSF

INTRODUCTION

Cerebrospinal Fluid (CSF) cytology is principally useful in the identification of malignant cells as the cytological changes in inflammatory and other non-neoplastic disorders are nonspecific. ^(1,2) It is an important part of a complete neurological evaluation of cancer patients, also important in patients with the acquired immunodeficiency syndrome (AIDS) particularly with neurological symptoms and patients with space occupying lesions (SOL) of brain. It is an essential step in the follow-up of patients with lymphoma and leukemia, round cell tumor and small cell tumor like small cell carcinoma of lung.

In recent era of newer therapeutic regimens and advanced diagnostic techniques, survival of cancer patient increase and so, incidence of Leptomeningeal carcinomatosis (LC) also

increases. Clinically one of the differential diagnoses of LC is infectious meningitis or encephalitis. Diagnosis of LC was given following the identification of malignant cells in the CSF. Diffuse or multifocal seeding of the leptomeninges by metastatic cancer cells was first reported by Eberth in 1870, ⁽³⁾ and the term 'carcinomatous meningitis' (CM) was introduced by Beerman in 1912. ⁽⁴⁾ It is previously known as CM, a devastating neurological complication of cancer; occurring in 3-8% of all cancer patients. ⁽⁵⁾ It arises from haematological malignancies or from solid intracranial or extra cranial tumors.

Useful tests to establish diagnosis of LC include magnetic resonance imaging (MRI), particularly gadolinium enhanced MRI (gd-MRI) study of the brain and spine, CSF cytology and radioisotope CSF flow studies. ^(6,7) Among all these diagnostic tests, CSF cytology is the only examination that

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verifies the presence of malignancy. A number of studies have proven that the cytological identification of malignant cells in the CSF remains the gold standard test for LM. (7,8,9,10)

The purpose of the study is to evaluate the importance of cytological examination of CSF as a useful and first line diagnostic technique for diagnosis of LM.

MATERIAL AND METHODS

The study includes retrospective analysis of patients who were admitted to MP Shah Cancer Hospital, Gujarat Cancer and Research Institute, the Cancer referral center of West India, between years 2007 to 2011. Total 5394 CSF specimens were collected during this five year period. Clinical data includes age and gender of patients, sign and symptoms, primary diagnosis, and follow up were noted.

Protocol for doing CSF examination at our hospital is as follows: Following newly diagnosed cases had CSF examination at first visit. Acute lymphoblastic leukemia (ALL), retinoblastoma (RB), medulloblastoma, Burkitts lymphoma, lymphoma in HIV positive patient, lymphoma with more than three extranodal sites involved, acute myeloblastic leukemia (AML)-myelomonocytic (M4) type and monoblastic (M5) type and AML with more than 1 lakh count. In remaining cases of malignancy, CSF examination was done when patient presented with clinical signs/ symptoms of meningitis or meningeal irritation during the course of disease.

1-2 ml of CSF collected by lumbar puncture (LP) method in EDTA vacutte. Processing of the specimen was done at cytology department. After physical examination: appearance, quantity and quality of CSF, centrifuge the specimen with cytocentrifuge (Cytospin-3) machine at 800 rpm speed for 3 minutes. A button was formed which provides more concentrated population of cells. Supernatant was preserved in another tube till the final report was given. For each case one slide was prepared. Papanicolaou stain was done by standard procedure. Whenever required, another slide was formed from the remaining CSF of that particular case. Samples of CSF for biochemistry were sent only in those cases when infectious etiology was suspected by clinician.

For suspicious CSF cytology, clinician repeat the CSF only if the patient was clinically normal, not showing any signs/ symptoms of meningeal irritation otherwise they considered it as positive and followed the patient for positive protocol of CSF cytology. For positive CSF cytology, intracranial RT and one cycle of intrathecal chemotherapy was given. Bi-weekly CSF cytology was sent. After three consecutive negative CSF reports, patient was transferred to his/her routine protocol of therapy. If the patient still remains positive for or had suspicious CSF cytology then second cycle of intrathecal chemotherapy was given.

RESULTS

Diagnoses were divided into positive cases, negative cases, suspicious for malignancy, hemorrhagic aspirate and having infectious etiology. a) Negative for malignant cells when smear was almost acellul or when few benign lymphocytes, monocytes or macrophages were seen in smear, b) suspicious for malignancy: when few atypical cells seen, in either paucicellular smear or in the background of reactive cells which were not conclusive for the diagnosis of malignancy, c) positive for involvement: when definite malignant cells seen.

In 5 years' period, total 5394 cases were sent to cytology department. In our study, patient's age was range from 1 to 70 years. In pediatric age group, male to female ratio was 3:1 and in remaining age group, it was 1.2:1. Out of 5394, 4985 cases were negative for malignant cells and 273 cases were positive for malignant cells, 110 cases were hemorrhagic, 26 cases were suspicious for malignancy and 4 cases had infectious etiology. Out of 26 suspicious cases, repeat CSF cytology was done in 18 cases in which 11 were become negative and 7 were positive, remaining 8 cases still showed signs/ symptoms of meningeal irritation so, they were treated as positive. Out of 288 positive cases, 153 were pediatric and 135 were adults. Distributions of cases were shown in Table-1. In four infectious etiologies, two had acute meningitis, one had Cryptococcus meningitis and last one had chronic meningitis. Out of four cases of SOL brain, one case had liver mass – further details not available, two cases suspected having GI malignancy, and last one was lost to follow-up without any investigation.

CSF cytology

In general, cytology of CSF revealed the general characteristics of the malignant cells consisted of cells with high nuclear : cytoplasmic (N/C) ratio, scanty to moderate cytoplasm, hyper chromatic nuclei, inhomogeneous or loosely clustered chromatin texture, prominent nucleoli, irregular nuclear membrane and presence of mitosis.

CSF involved by leukemia/lymphoma usually had discohesive, relatively monotonous cells with high N/C ratio, thin rim of cytoplasm, irregular nuclear membrane, and presence of nuclear protrusion or folding and large nucleoli. In some cases hand- mirror cells seen. Apoptotic bodies were also seen in few cases. CSF involved by chronic myeloid leukemia (CML), the cell population is heterogeneous, with all stages of granulocytic precursors with or without increase number of blast cells. Retinoblastoma, medulloblastoma and Peripheral neuroectodermal tumor (PNET) were morphologically similar in CSF. In all cases, cells are usually numerous and identified as malignant. The cells vary in size, relatively monotonous, occurs singly, in small clusters or rosettes. The

cytoplasm is scanty and nuclei are hyperchromatic. Nuclear molding and apoptotic bodies were seen in many cases. The cytology in CSF of metastatic cancer depends on the type of tumor. (Figure-1 and Figure-2) The recognition of the organ of origin or even tumor type may be extremely difficult in cytological preparation in the absence of clinical history. In general, cells of metastatic carcinomas, even of small size, are larger than transformed lymphocytes.

DISCUSSION

Malignant cells infiltrate the leptomeninges or the CSF cavity by direct extension or by haematogenous spread or by lymphatic metastasis. ^(11, 12) Misdiagnosis of LC occurs frequently because of its diverse clinical symptoms and its association with high mortality and major neurological disability. ⁽⁵⁾ So, it is of great importance to recognize its presentation and to improve the diagnosis. Lymphoma, leukemia and some carcinoma particularly small cell carcinoma of lung may be associated with treatable occult cerebrospinal metastasis. Early recognition of this metastasis may be critical in deciding on further therapeutic measures that may significantly alter the course of the disease.

One of the useful tests to establish diagnosis of LM is gd-MRI but it showed an approximately 30% incidence of false negative results and so negative imaging does not exclude the diagnosis of neoplastic meningitis (NM).⁽¹³⁾ CSF analysis could represent a valid method for diagnosis of NM because of direct demonstration of tumor cells among all these diagnostic tests.

Even in lesion that are in contact with CSF, the number of malignant cells observed may be very small, so even a single abnormal cells should be most carefully evaluated as it may prove to be of diagnostic value. The use of liquid based monolayer technology seems to overcome most of these diagnostic difficulties. This procedure provides better cytomorphology, higher cellularity per slide, clean background with easier detection of malignant cells and reproducibility of equivalent shades for immunocytochemistry. Sioutopoulos Do et al. ⁽¹⁴⁾ suggested thin preparation is as an alternative method of preparation for CSF specimen. Only in malignant lymphoma and leukemia and rarely in other metastatic tumor, abundant cancer cells consistently present in CSF. The CSF cytology of metastatic cancer depends on the type of primary tumor.

In our study commonest malignancy found in CSF was acute leukemia in both adult and pediatric cases, account for 44.5% and 67.5% of cases respectively. Lymphoblastic leukemia was more common in both age groups. In pediatric series ALL is 52.9% followed by Retinoblastoma (25.5 %). Our results correlate well with the study of Payerson et al. ⁽¹⁵⁾

Lymphoma accounts 11.7% of cases followed by AML and Medulloblastoma involving similar number of cases (4.7%). In adult, leukemia (44.5%) was followed by epithelial malignancy (28.1%) and lymphoma (22.9%) in positive CSF cytology. In epithelial malignancy most common involvement is by breast carcinoma (16.3%) followed by lung cancer (6.7%). Other sites included were ovary, cervix, colon, and stomach. Two cases of Medulloblastoma were also found in adult. It has been reported in the literature that the most common malignancies associated with LC are breast cancer, lung cancer, melanoma, lymphoma and leukemia.^(16,17) Our results were comparable to other studies.^(3,4,14,18,19,20,21) All cases of lymphoma involving CSF in our study had systemic spread rather than primary CNS lymphoma. In pediatric, out of 18 positive cases of metastatic NHL, 15 were which lymphoblastic lymphoma and 3 cases of T-cell lymphoma. In adult, out of 31 positive cases, 16 were diffuse large B-cell lymphoma, 8 cases were lymphoblastic lymphoma, 3 were T cell lymphoma, 2 were B cell lymphoma and 2 were Burkitt's lymphoma. Amongst the breast carcinomas, most of our cases were duct type, only one case was lobular type and in lung carcinoma most cases were adenocarcinoma. Our results correlate with the study of Chuang TY et al. ⁽²²⁾ but it contrast to other studies. ^(23, 24, 25)

Now a day, CSF examination is a routine procedure in acute leukemia of children. It has been shown that leukemic cells may be present in CSF in asymptomatic patients with good response to the therapy, before there are clinical manifestations of meningeal involvement. Thus, CSF cytology serves to monitor the effect of treatment in sequential samples of CSF, particular in children. ^(26, 27)

Several sources of errors in diagnosis of leukemia, lymphoma in CSF were identified like viral and fungal meningitis, viral encephalitis, contamination with peripheral blood in a known case of leukemia and a postsurgical reaction. The errors are avoided by following the strict morphologic criteria of CSF. In case of doubt, additional material should be requested and processed by immunocytochemistry and flow cytometry.

Our results suggest that CSF cytology is helpful in the diagnosis of tumor metastasis to leptomeninges. CSF metastasis correlates with worse prognosis and patient survival. Treatment in broad prospective entails radiotherapy (intrathecal) and Systemic chemotherapy.⁽²⁸⁾ The clinical value of an early and accurate diagnosis of metastatic tumors in CSF is high even in neurologically asymptomatic patients who are in remission from neoplasms that were formerly rapidly fatal, because the effect of treatment contributes to quality of life, control of neurological symptoms and longer survival. Since the CNS is outside the field of radiation and many chemotherapy agents cannot penetrate the blood brain barrier, these patients may present with relapse in the CNS.

Since long, detection of neoplastic cells by cytological examination of CSF has become commonplace in most cytopathology laboratories. This technique will still remain the first-line diagnostic procedure in patients with metastatic tumors, leukemia, lymphoma and primary brain tumors who are suspected of having meningeal spread as well as in patients who present initially with signs of meningeal disease of unknown etiology.

CONCLUSION

The present case series also states that cytological examination of CSF is simple and useful technique even possible at primary health centre still remains the first line diagnostic technique for diagnosis of LM. CSF analysis could also represent a valid method for diagnosis of neoplastic meningitis because of direct demonstration of tumor cells among all other diagnostic tests.

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Note: In the present study, CSF aspiration procedure performed on the patient as routine diagnostic procedure which was with prior consent of the patients. Ethical committee clearance has not been required as confidentiality of patients' details has not been published.

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Abbreviation

Cerebrospinal fluid (CSF)

Leptomeningeal carcinomatosis (LC).

Acquired immunodeficiency syndrome (AIDS)

Space occupying lesions (SOL)

Acute lymphoblastic leukemia (ALL),

Retinoblastoma (RB),

Acute myeloblastic leukemia (AML)

Myelomonocytic (M4) type

Monoblastic (M5) type

Lumbar puncture (LP)

Peripheral neuroectodermal tumor (PNET)

Chronic myeloid leukemia (CML),

Neoplastic meningitis (NM)

Gadolinium enhanced MRI (gd-MRI)

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Table 1: Distribution of positive cytology cases with their primary site of origin

Tumor type	Pediatric Cases N=153(%)	Adult Cases N=135(%)
Acute Lymphoblastic Leukemia	81(52.8)	48(35.6)
Acute Myeloid Leukemia	7(4.7)	5(3.7)
Retinoblastoma	39(25.5)	-----
Nonhodgkinlymphoma	18(11.7)	31(22.9)
Medulloblastoma	7(4.7)	2(1.5)
PNET	1(0.6)	-----
Chronic myeloid leukemia	-----	7(5.2)
Carcinoma Breast	-----	22(16.4)
Ca Lung	-----	9(6.6)
Ca Ovary	-----	4(2.9)
Carcinoma Cervix , carcinoma colon, carcinoma stomach	---	1 (0.7) each
SOL Brain	-----	4(2.9)

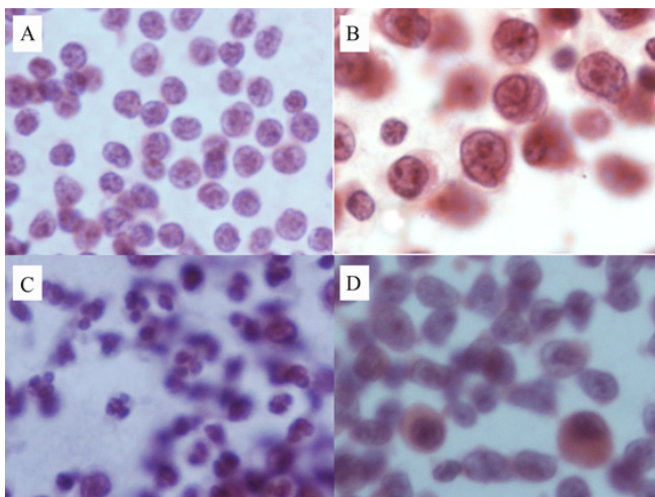


Figure 1:
A. ALL Microphotograph showing discohesive, relatively monotonous cells with high N:C ratio, thin rim of cytoplasm,

irregular nuclear membrane, presence of nuclear protrusion or folding and large nucleoli. (PAP,100X)

B. DLBCL Microphotograph showing large cells with irregular nuclear membrane and prominent nucleoli. (PAP, 100X)

C. Acute meningitis Microphotograph showing polymorphs admixed with small lymphocyte and macrophage. (PAP, 100X)

D. CML Microphotograph showing is heterogenous cell population, with granulocytic precursors- myelocyte with increase number of blast cells. (PAP, 100X)

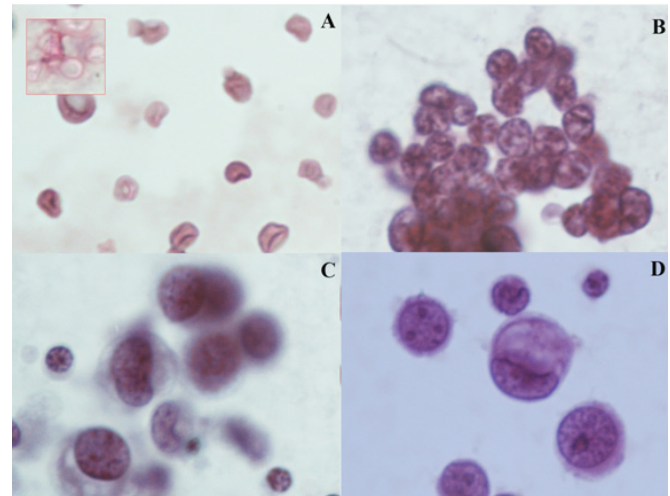


Figure 2:
A. Cryptococcus Microphotograph showing the characteristically thick capsule of cryptococcus. (PAP, 100X) Inset showing mucicarmine stain.(100X)
B. RB Microphotograph showing small clusters of malignant round cells with scanty cytoplasm and hyperchromatic nuclei. (PAP, 100X)
C. Metastatic lung adenocarcinoma Microphotograph showing loose cluster of large cells with pale or vacuolated cytoplasm. Lymphocyte was seen along with for comparison. (PAP, 100X)
D. Metastatic ductal carcinoma Microphotograph showing single large cell, vesicular nuclei pushed by mucin vacuole in the cytoplasm. Lymphocyte was seen along with for comparison. (PAP, 100X)