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Original Article

Pathology Section

Utility of Intraoperative Imprint Cytology in Diagnosis and Grading of Glioma: A Cross-sectional Study

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ABSTRACT

Introduction: Gliomas are most common primary Central Nervous System (CNS) neoplasm in adult population. Gliomas predominantly arise from brain parenchyma. Invasion of adjacent normal parenchyma is a prominent feature.

Aim: To study the epidemiological incidence of glial tumours and the viability and accuracy of intraoperative cytology in diagnosis and grading glial tumours.

Materials and Methods: A cross-sectional study was done on 30 patients who underwent excision surgery at Department of Neurosurgery at Institute of Postgraduate Medical Education and Research IPGMER and SSKM, Kolkata, West Bengal, India. Clinically, radiologically confirmed cases of Space Occupying Lesion (SOL) of brain with a history of neurosurgical intervention were included. Patients medically unfit for surgery or without radiological evidence of SOL in the brain were excluded from the study. Intraoperative imprint touch cytology of the specimens was done. Part of the tissue was kept for Formalin Fixation and Paraffin Embedding (FFPE) and subsequent histopathological examination were done. Results of intraoperative imprint cytology were compared with final histopathology report and grading. Matthews correlation coefficient t-test, Kohen's Kappa (κ), Chi-square test (χ^2) were used for the statistical analysis. A p-value of $p<0.5$ was considered statistically significant.

Results: About 10 (33.3%) of the patients presented with frontal lobe lesion. Out of 30 cases 29 (96.6%) were histologically confirmed to be of glial origin, 1 (3.3%) was metastasis from other tumour. These 29 cases were diagnosed as different glial neoplasm on intraoperative imprint cytology and were confirmed by histology. According to histological subtype 12 (41.37%) were glioblastoma, 10 (34.48%) were diffuse astrocytoma, 3 (10.34%) were pilocytic astrocytoma, 3 (10.34%) was ependymoma. Intraoperative impression cytology diagnosis was compared with confirmatory histological diagnosis. Sensitivity and specificity was found to be 93% and 50%, respectively, Positive Predictive Values (PPV) 96%, Negative Predictive Values (NPV) 33%, diagnostic accuracy 89.65% and $p<0.5$ was statistically significant.

Conclusion: In the present study, confirmatory histopathology report showed a significant association with imprint cytology report. Hence, it can be concluded that intraoperative imprint cytology is a fairly accurate, rapid and inexpensive method of diagnosis and grading of gliomas.

Keywords: Central nervous system, Diagnostic accuracy, Glial tumours, Histopathology, Intraoperative

INTRODUCTION

Gliomas are the most common primary neoplasm in the adult population. About 40% of all the intracranial neoplasms originate from neuroepithelium, 35% from meningothelial cells, 14.4% from oral ectoderm and 7.5% from peripheral nerve sheath elements. Lymphomas and germ cell tumours are rare and only make upto 2.3 and 0.5%, respectively [1]. Approximately, 30% of all the neoplasms of brain are glioma, among them 75% gliomas are astrocytoma [2]. Histological spectrum of glioma consists of nuclear atypia, mitotic activity, microvascular proliferations, tumour necrosis, grade of the tumour depends on various combinations of these factors [3]. Grading of tumour is of crucial importance from the therapeutic aspect, because the course of chemotherapy and radiotherapy is dependent on it [4]. It has been reported in past that squash cytology alone can be used to analyse cytomorphology of glioma cells, including nuclear morphology, coarse chromatin pattern, mitosis, tumour necrosis thus differentiating low grade and high grade gliomas [5-10]. Thus, a correct understanding of the biologic behaviour during the surgical intervention shall positively influence the outcome.

The study was undertaken to evaluate the epidemiological occurrence of glial tumour and to ascertain the validity and reliability of intraoperative diagnosis of glial tumour and correlate with subsequent histopathological diagnosis and to ascertain the subtyping and grading of glial tumour with respect to current World Health Organisation (WHO) classification of tumours of central nervous system [3].

MATERIALS AND METHODS

This present cross-sectional study was conducted from August of 2020 to August of 2021 at IPGMER and SSKM Hospital Kolkata in the Department of Pathology on the specimens received from 30 patients who underwent excision surgery at Department of Neurosurgery. The study was performed after obtaining the approval from Ethical Committee (IPGME&R/IEC/2021/019). The patients presenting with headache, vomiting, convulsion came to Department of Neurosurgery for evaluation. Radiological investigations like Computerised Axial Tomography (CAT) and Magnetic Resonance Imaging (MRI) scan was done. Upon locating intracranial SOL surgical intervention was planned. During the surgeries intraoperative imprint cytologic preparation were performed to understand the exact nature of the neoplasms.

Inclusion criteria: Patients diagnosed with neoplasms of glial origin were included in the study.

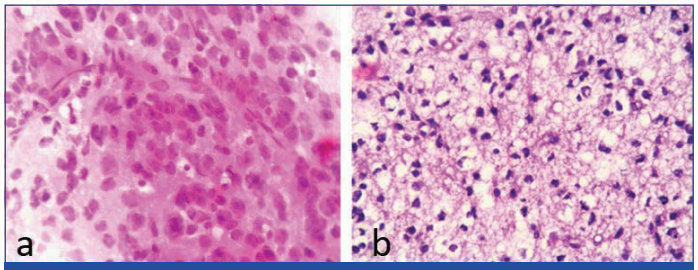
Exclusion criteria: Cases diagnosed as neoplasms other than glioma were excluded.

The specimens were sent to the Department of Pathology for further examination.

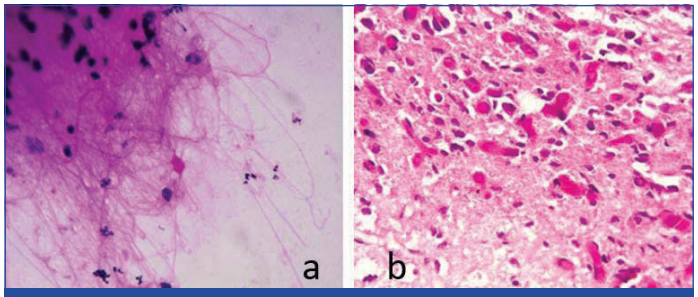
Study Procedure

Imprint smears preparation and cytological examination: In all the cases, intraoperative tumour tissue pieces were received from neurosurgery. Fragments from the tissue fragments were touched with clean glass slides with mild pressure to properly spread the cellular material after blotting excess blood. After air drying and

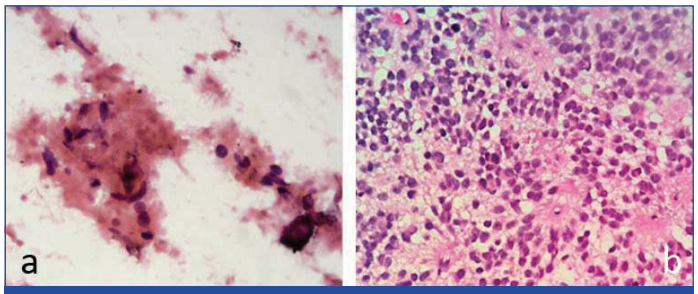
fixation in 95% ethyl alcohol rapid Haematoxylin and Eosin staining (H&E) was done in imprint cytology smear and provisional diagnosis was given. The following morphologic features are observed for diagnosis and grading. Cellularity of the smear, shape of the cell nuclei (round, oval or spindle), nuclear atypia (to ascertain the grade and to differentiate neoplastic glial cells from gliosis), chromatin pattern (typical coarse and knobby chromatin of glial neoplasm), nuclear enlargement (disproportionately large cells signifies higher grade), cellular pleomorphism (degree of pleomorphism corroborates well with malignant nature), mitosis (number of mitosis per 10 HPF is crucial in asserting the grade). Tumour necrosis (present or absent), vascular proliferation and vessel morphology. A part of the tissue was preserved in formalin for paraffin embedding and histopathological examination. The histological subtyping and grading were done according to recent WHO classification of CNS to reach a confirmed diagnosis and prediction of accuracy of imprint analysis [Table/Fig-1-4]. Due to heterogeneity of CNS neoplasms and inadequacy in tissue sampling, there is difficulty in assessing grades and subtypes of gliomas in imprint smear. The evidence of brain invasion is not always apparent in imprint smears. Very often there is a downgrade of histological grade in imprint smear.



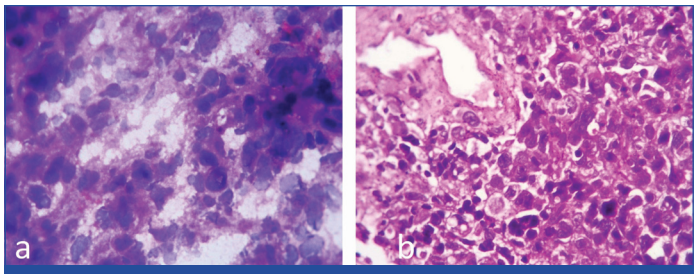
[Table/Fig-1]: a) A imprint cytology of diffuse glioma x400; b) Histological picture of diffuse glioma, x400 (H&E).



[Table/Fig-2]: a) Rosenthal fibres in imprint cytology of pilocytic astrocytoma x100; b) Histological picture of pilocytic astrocytoma, x400 (H&E).



[Table/Fig-3]: a) Imprint Cytology of Anaplastic ependymoma, x100; b) Histological picture of Anaplastic ependymoma, x400 (H&E).



[Table/Fig-4]: a) Imprint cytology of glioblastoma. x400; b) Histological picture of of glioblastoma, x400 (H&E).

STATISTICAL ANALYSIS

All data was copied into Microsoft Excel and contingency charts were prepared for qualitative data. Data were analysed with the help of Statistical Package of the Social Sciences (SPSS) software version 20.0 (IBM, Armonk, New York,USA), Prism Graph Pad version 5. Matthews correlation coefficient t-test, Kohen's Kappa (κ), Chi-square test (χ^2) were used for the statistical analysis. $p<0.5$ was considered statistically important.

RESULTS

The incidence of this tumors in this institute is approximately about 30 per year. Out of 30 patients 16 (53.3%) were males and 14 (46.6%) were females. Age of the patients was in the range of 3-80 years at the time of surgery. Mean age was 36.3±3.817. There was five patients within the age group of 20 years, 11 patients within 21-40 years, 11 patients within 41-60 years and four patients within 61-80 years. As per site of occurrence most of them 10 (33.3%) were in the frontal lobe, 5 (16.7%) were from the parietal lobe, 1 (3.33%) from frontoparietal lobe 5 (16.6%) were from temporoparietal lobe, 3 (10%) were from temporal lobe, 2 (6.66%) from posterior fossa 2 (6.66%) from parietooccipital lobe 1 (3.33%) from thalamus and 1 (3.33%) from frontotemporal lobe [Table/Fig-5].

Location	Number
Frontal	10
Parietal	05
Frontoparietal	01
Temporal	03
Temporoparietal	05
Posterior Fossa	02
Thalamus	01
Parietooccipital	02
Frontotemporal	01

[Table/Fig-5]: Distribution of site of total tumours.

The histological type of glioma was ascertained as per WHO 2016 CNS tumour classification. Most of the tumour 12 (41.37%), were glioblastoma followed by diffuse astrocytoma 10 (34.48%), pilocytic astrocytoma 3 (10.34%) and ependymoma 3 (10.34%). One case was metastastic deposit. The spectrum of histological grade of these 29 glioma cases was varied. Majority of the tumours (13/29) were WHO Grade IV followed by WHO Grade II (12/29), WHO Grade I (3/29), WHO Grade III (1/29). Out of 30 cases, 29 cases were histologically confirmed as glioma, one was metastatic deposit, so diagnostic accuracy was 89.65%. The correlation of intraoperative impression cytology and histological diagnosis were analysed and it was found to have sensitivity of 93%, specificity of 50%, PPV of 96%, NPV of 33%, false positive ratio (alpha) 50%, false negative ratio (beta) of 7%, positive likelihood ratio 1.86, negative likelihood ratio of 0.14 [Table/Fig-6].

Variables	Value
Sensitivity	93%
Specificity	50%
PPV	96%
NPV	33%
False positive rate (α)	50%
False negative rate (β)	7%
Positive likelihood ratio	1.86
Negative likelihood ratio	0.14

[Table/Fig-6]: Overall efficacy of intraoperative imprint cytology in diagnosis of glioma.

DISCUSSION

Intraoperative imprint cytology, in some aspects superior to frozen sections for rapid observation and assessment of cellular morphology and subsequent diagnosis and grading. Along with cellular morphology

other factors like clinical profile, neuroradiological examination are cardinal for an accurate diagnosis. Intraoperative consultation with the pathologist assists the surgeon in deciding the method of surgery and subsequent follow-up and management. In a study by Fujita H et al., cellular morphology can clearly differentiate between low grade glioma and high-grade glioma and vascular morphology of squash preparation of astrocytoma can be useful in asserting the grade of the tumour [11]. In a study with 63 patients, Samal S et al., showed complete correlation. Diagnostic accuracy of cytology for glialtumours were 88.24%. The sensitivity, specificity, positive predictive value in detecting neoplastic condition were 94.4%, 85.7%, 98.07% and 66.67%, respectively [12]. Another study by Sharma S and Deb P with 90 cases showed that in case of pilocytic astrocytoma, ganglioglioma, glioblastoma and ependymoma diagnostic accuracy reached 100%, in case of low-grade glioma the diagnostic accuracy was 71.4% and only 50% accuracy in high grade glioma as one out of two cases was misdiagnosed as high-grade gliomas [13]. In this study, there were 30 cases, among them 29 were glial tumour and one came out to be metastatic deposit in the brain. Mean age was 36 with a mild female preponderance. Frontal lobe was the most common site. The most common histological type was glioblastoma. In 26 cases intraoperative cytological diagnosis was corroborated with histopathological diagnosis. One case of anaplastic ependymoma was initially diagnosed as glioblastoma in intraoperative consultation. Another case of oligodendroglioma was diagnosed as diffuse astrocytoma in imprint cytology. A case of midline glioma was initially identified as glioblastoma intraoperatively. A case of metastasis to brain was initially identified in cytologic preparation and was confirmed by histopathology. Grade of the tumour was correctly predicted by imprint cytology in most of the cases A sensitivity level of 93%, PPV 96% and lower specificity of 50%, NPV of 33% was calculated in the present study. Hence, imprint cytology was found to be a highly sensitive process with a low specificity. As the results of the present study were compared with with studies in the past, they were found to be corroborative. Cellularity of a smear often indicates the grade of the tumour, higher grade tumour tends to demonstrate highly cellular smears, but the cellularity depends on the procedure of smear preparation and the quality of the sample. As in the present study a case of anaplastic ependymoma had a smear with scanty cells. Individual cellular and nuclear morphology is of paramount importance. Large cells along with cellular pleomorphism in smear corroborates well with the histopathological diagnosis and grading. Morphology of the chromatin plays an important role. Coarse and knobby chromatin with fibrillary background points to glial origin.

Tumour necrosis is an important factor in the diagnosis of glial tumour of higher grade. Homogeneous eosinophilic necrotic areas are seen in case of glioblastoma. Piloid changes like rosenthal fibres on an imprint cytology smear are seen in case of pilocytic astrocytoma. As per previous studies minimum of four core biopsy specimens could be essential for the highest diagnostic yield (89%) [14,15]. Samples from high risk areas like brain stem are often scanty in amount. In these situations, only a single suction aspiration biopsy may be performed usually permitting the application of only a single touch preparation. It has been suggested in the past that in such cases, the biopsy tissue is to be fixed in Karnovsky's solution for plastic embedding and subsequent electron microscopic evaluation [16].

Limitation(s)

Limitations of this study have been the lack of genomic study of these tumours. In the time period, 30 cases were collected, a larger sample size could have provided a better statistical results.

CONCLUSION(S)

Hence, it can be concluded here that intraoperative imprint cytology is a reasonable, fairly accurate, rapid and inexpensive procedure as far as the diagnosis of tumours of glial origin is concerned. In the present study it is clear that imprint cytology is a process with high sensitivity and low specificity. Timely and precise opinion of a neuropathologist in the intraoperative period helps to guide the course of treatment to the right direction.

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