<u>**Original Research Article**</u>

The Utility of Intraoperative Squash Cytology and its Diagnostic Accuracy in Central Nervous System Tumours with Expression of IDH1 in Selected Cases of Glioma

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Abstract:

Background: Annually, the incidence of Central Nervous System tumours range from 10-17 per 1,00,000 population among all the intracranial tumours. Intraoperative squash cytology is a rapid, inexpensive, non-invasive and fairly accurate procedure for diagnosing Central Nervous System tumours.

Aims and Objectives: To establish the utility of squash cytology and its diagnostic accuracy in Central Nervous System tumours and its histopathological correlation with expression of Isocitrate Dehydrogenase 1 status in cases of glioma.

Materials and Methods: A total of 52 cases of CNS tumours suspected clinical-radiologically were taken into consideration. Intraoperative squash cytology was prepared and the rest of the tissue was sent for histopathological and immunohistochemical evaluation. Immunohistochemical staining of Isocitrate Dehydrogenase 1 was performed for histopathologically diagnosed gliomas. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy was calculated.

Results: The overall diagnostic accuracy was found to be 98.7% for all the Central Nervous System tumours and expression of IDH1 was seen in 11 cases of glioma.

Introduction

Intraoperative squash cytology (ISC) is an established method of diagnosing CNS tumours. It's a rapid, fast and inexpensive procedure. As CNS tumours are vast and diverse, it is quite challenging to diagnose accurately and providing an intraoperative diagnosis is of utmost importance as it helps neurosurgeons in optimizing surgical procedures.

The incidence of CNS tumours range from 10-17 per 1,00,000 population among the intracranial tumours [1,2]. According to the 5th edition of WHO 2021 classification, CNS tumours are divided broadly into : (1) Adult-type diffuse gliomas, (2) Paediatric-type diffuse low-grade gliomas, (3) Paediatric-type diffuse high grade gliomas, (4) Circumscribed astrocytic gliomas, (5) Glioneuronal and Neuronal tumours (6) Ependymal tumours (7) Embryonal tumours (8) Pineal tumours (9) Cranial and paraspinal nerve tumours (10) Meningiomas (11) Mesenchymal, non-meningothelial tumours (12) Haematolymphoid tumours (13) Histiocytic tumours (14) Germ cell tumours (15) Tumours of the sellar region and (16)

Metastases to the CNS [3]. Isocitrate dehydrogenase (IDH) enzymes, has three isoforms, are important enzymes which participate in many major metabolic processes, like the Krebs cycle, lipogenesis, redox regulation and glutamine metabolism.

In glioma, around 80% of IDH mutations are present in World Health Organisation (WHO) grade 2/3 cases.

IDH-mutated glioma shows a better disease outcome. However, increased incidence of IDH mutations in secondary grade 4 glioma shows that lower-grade glioma with IDH mutation have increased recurrence following malignant transformation to a higher grade. Also, IDH-mutated glioma develops a hypermutation phenotype, which shows a worse prognosis [4,5].

This study was undertaken to establish the utility and diagnostic accuracy of intraoperative squash cytology in gliomas and glioneuronal tumours with IHC status of IDH1 in cases of glioma.

Materials and Methods

A hospital based cross sectional study was carried out for a period of one year from June 2021 to July 2022 in the Department of Pathology, Gauhati Medical College and Hospital. Detailed clinical history of the patients such as presenting symptoms, location of lesion, and radiological findings were noted. All suspected cases of CNS tumours on radiology which fulfilled our inclusion and exclusion criteria were taken into consideration. Inadequate biopsy samples were excluded from the study.

One to two millimetres of biopsy material was placed at one edge of clean, dry and labelled slide and smeared with another slide. The smears were fixed in 100% isopropyl alcohol rapidly and Haematoxylin and eosin staining was performed. The rest of the tissue was fixed in 10% buffered formalin and processed for histopathological evaluation. In total of 17 cases which were diagnosed as gliomas, IDH1 immunohistochemistry was performed and recorded. A cytoplasmic and perinuclear immunoreactions for IDH1 were scored as positive.

Squash cytology and histopathological slides were reviewed by two different senior pathologists. Discordant cases were evaluated and recorded. The cases were divided into true negative (absence of tumour correctly diagnosed); true positive (presence of tumour correctly diagnosed); false negative (the cytological specimen failed to diagnose as tumour); and false positive (the cytological specimen was incorrectly diagnosed as tumour). Diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value was calculated and arranged in 2*2 table.

Results and Observation

A total of 52 cases fulfilling our inclusion and exclusion criteria were taken into consideration. The age range was between 2-70 years. The median age was 33 years. The highest amount of cases were between the age group of 21-40 years. 82.6% (n=43) of patients were in the adult age group and rest belonged to paediatric age group which were 17.3% (n=9) cases. Male to female ratio was 1.36:1 with preponderance of male cases 57.6% (n=30).Out of the 52 cases, 11.8% (n=2) of the cases were non-neoplastic and the rest of the cases were neoplastic 96.15% (n=50). Most of the patients presented with headache, vomiting, seizures followed by paresis. The paediatric cases came with complaints of increased intracranial pressure. The percentage of various primary CNS neoplastic lesions is shown in Figure 1 and the data showing sensitivity, specificity, positive predictive value , negative predictive value and diagnostic accuracy are enumerated in table 1.
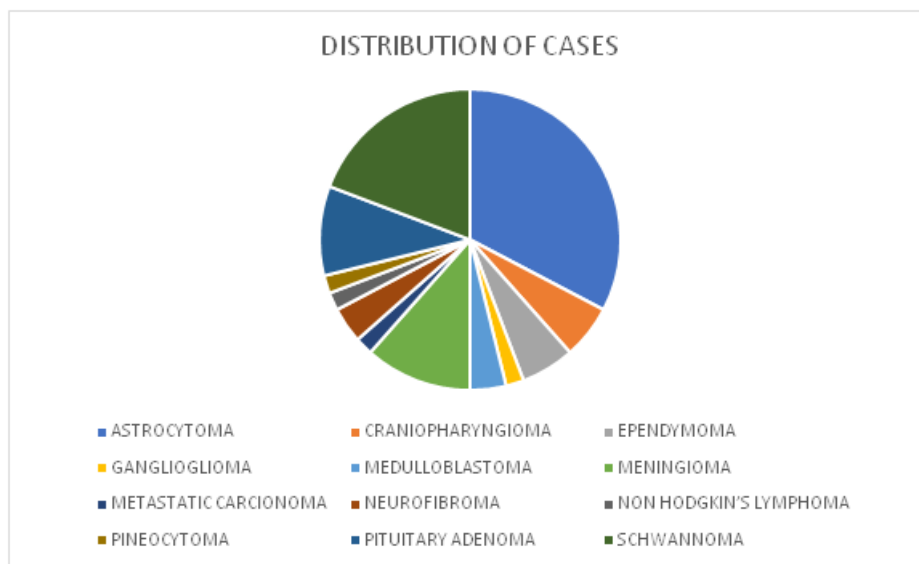

Figure 1: Data showing the spectrum of neoplastic lesions

Table 1: Data showing sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy

Histopath olgical Diagnosis	Corrrect Cytologic al Diagnosis	Total No. Of Cases	Discordan t Cases	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Accuracy
Astrocytoma	15	17	2	89.40%	100%	100%	94.50%	96.20%
Craniopharyngioma	3	3	0	100%	100%	100%	100%	100%

Ependymoma	2	3	1	75%	100%	100%	96%	98.10%
Ganglioglioma	1	1	0	100%	100%	100%	100%	100%
Medulloblastoma	2	2	0	100%	100%	100%	100%	100%
Meningioma	5	6	1	85.70%	100%	100%	97.80%	98.10%
Metastatic Carcinoma	1	1	0	100%	100%	100%	100%	100%
Neurofibroma	2	2	0	100%	100%	100%	100%	100%
Non-Hodgkin's Lymphoma	1	1	0	100%	100%	100%	100%	100%
Pineocytoma	1	1	0	100%	100%	100%	100%	100%
Pituitary Adenoma	4	5	1	83.30%	100%	100%	97.90%	98.10%
Schwannoma	8	10	2	76.90%	100%	76.90%	93.30%	94.50%
Total	45	52	7	92.50%	100%	92.50%	92.50%	98.70%

Discussion

Among 52 cases, 32.6% (n=17) cases were astrocytoma which were categorised into low grade and high grade. Among the 17 cases, 15 cases were correctly diagnosed on squash cytology. Astrocytoma was the most common CNS tumour in our study with a diagnostic accuracy of 96.2% which is similar to other studies. Low grade glioma showed low to moderate cellularity with oval to elongated nuclei surrounded by pale staining cytoplasmic processes. (Figure 1a) which was confirmed on histopathology (Figure 1b,1c).

High-grade gliomas showed increased cellularity and pleomorphic cells with areas of necrosis and endothelial proliferation.
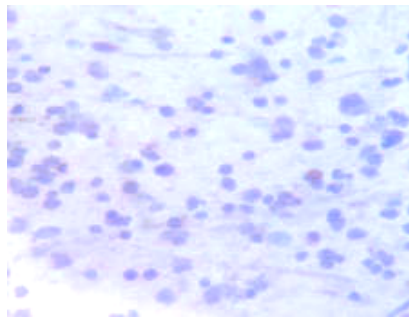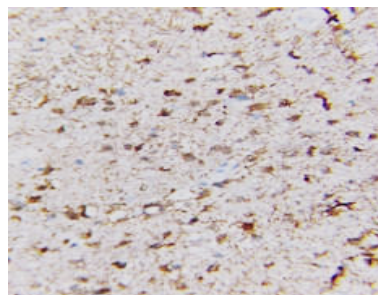

2a

Figure 2a: Squash smear showing low grade astrocytoma (H&E, 40x)
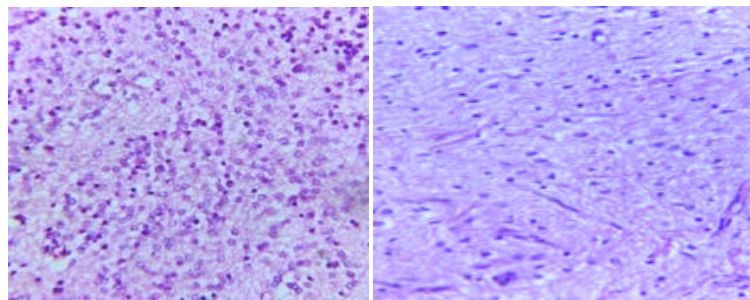
2b   2c

Figure 2b and 2c: Photomicrograph showing grade 2 astrocytoma on histopathology (H&E, 40x)

2d

Figure 2d: Photomicrograph showing immunohistochemistry of IDH1 in tumour cells

One case of squash cytology of astrocytoma was misdiagnosed as ependymoma due to similarity of perivascular astrocytic processes and ependymal rosettes. Ependymomas constituted 5.7% (n= 3) cases with a diagnostic accuracy of 98.1%.One case of Ependymoma was diagnosed as astrocytoma on squash cytology due to the absence of ependymal rosettes. Schwannoma constituted 19.2% (n=10) cases with a diagnostic accuracy of 94.5%. Two cases of schwannoma were misdiagnosed on squash cytology. One case was given as meningioma and another was deemed as unsatisfactory smear for diagnostic opinion.

Smearing was difficult and cytology showed areas of spindle cells in cases of schwanomma. One case of pituitary adenoma was misdiagnosed as oligodendroglioma with a diagnostic accuracy of 98.1%. Pituitary adenoma constituted 9.6% (n=5) cases. The rest of the cases were Craniopharyngioma, Ganglioglioma, Medulloblastoma, Metastatic carcinoma, Neurofibroma, Non-Hodgkin's Lymphoma, Pineocytoma which showed no discordance with a diagnostic accuracy of 100%. Comparison of diagnostic accuracy of different studies with present study is shown in Table 2.

Table 2: Comparison of diagnostic accuracy of different studies with present study

Studies	Diagnostic Accuracy
Philip et al [6]	93.8%
Jindal et al [7]	94.67%
Goel et al [8]	85%
Shukla et al [9]	87.76%
Present study	98.7%

IDH1 immunoexpression was seen in 64.7% (n=11) cases of astrocytoma which were grade 2 to grade 4. Five cases (65.4%) of grade 2 glioma, 4 cases (100%) of grade 3 glioma and 2 cases of grade 4 (85.4%) glioma showed IDH1 positivity by immunohistochemistry (Figure 1d). This is similar to studies done by Mellai et al and Ayad et al [10,11].

Conclusion

Intraoperative squash cytology is of utmost importance as its diagnostic accuracy in different studies range from 80-100%. It is an ideal method which is rapid, inexpensive, accurate and simple. However, a proper history with radiological findings helps pathologist to come to an accurate diagnosis.

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