Value of Touch Preparation Cytology in Intraoperative Consultation Diagnosis of Astrocytomas

Humaira Nasir and Anwar Ul Haque

Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad.

Intra-operative diagnosis of brain tumours routinely employs frozen sections. The frozen section however has several limitations and induces serious freezing artefacts. We carried out a prospective study on 40 cases of astrocytomas utilizing touch preparation cytology. The smears were quite cellular with crisp cellular details. There was 98.4% sensitivity and 90% specificity. We found touch preparation cytology highly reliable mode of intra-operative diagnosis in astrocytomas.

Keywords: Gliomas, Astrocytomas, Touch Preparation Cytology, Frozen Section.

Introduction

Brain tumours constitute about 10% of all tumours, and glial neoplasia account for around 70% of them.1 The astrocytomas of the cerebral hemisphere are currently classified into three tiers: well-differentiated astrocytoma, anaplastic astrocytoma and glioblastoma multiforme. In addition, special forms of astrocytoma are also recognized, including juvenile pilocytic astrocytoma and fibrillary astrocytoma.

Low-Grade Astrocytoma is characterized by slight hypercellularity, mild nuclear enlargement, slight pleomorphism, and absence of mitotic activity, endothelial proliferation and necrosis. The astrocytes are separated by glial fibres. The astrocytic nuclei show mild pleomorphism with frequent angulation and irregularity. When round oligodendroglial cells are present in large numbers, the diagnosis of mixed oligoastrocytoma must be considered.2

Anaplastic astrocytoma is characterized by the presence of significant hypercellularity, marked cytologic atypia and mitotic figures. Glioblastoma multiforme (GBM) displays prominent vascular growth, endothelial cell proliferation, mitoses, markedly dysplastic nuclei, prominently varied patterns and necrosis. The endothelial cells are proliferated in response to focal thrombus formation. These may assume papillary, tufting or glomerular pattern. These cells may appear as cohesive syncitial clumps or in a linear fashion interspersed with neoplastic astrocytes.

Intra-operative diagnosis of brain tumours entails frozen section and intra-operative cytology, both of which provide rapid intra-operative pathologic consultation to the neurosurgeons.3 Cytology samples much wider areas, which is important as astrocytomas may display different grades in different areas and at different depths. Further, it is easier to obtain an excellent touch preparation cytology than to have a high quality brain tissue frozen section. A good cytological preparation of brain displays high cellularity, crisp nuclear and cytoplasmic details and occasionally the tissue architecture.4 On the other hand, frozen sections on brain tissue may develop many artefacts including those produced by the ice crystals and water logging in the tissues. As a result, tissue often appears smudgy, foggy and shattered. Moreover, previously frozen brain tissue displays artefacts on paraffin sections.
On the other hand, all the tissue can be used for making touch preparation and afterwards entire tissue can be submitted for paraffin sectioning, etc. It eliminates freeze artefacts and markedly enhances the intra-operative consultation speed without compromising diagnostic accuracy. This strategy has proven to be highly sensitive and accurate. Many workers are now convinced that smears represent the most accurate and efficient way of diagnosing brain lesions. The aim of the present study was to evaluate the value of touch preparation cytology in intra-operative consultation diagnosis in cases of astrocytomas.

Patients and Methods

The study was conducted in the Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad from January 1999 to September 2001.

During the period of study, one hundred & thirty cases of various tumours of brain and spinal cord were diagnosed. Forty of these patients, who had astrocytoma were included in the study.

Preoperatively clinical information regarding age, gender, detailed clinical history and CT scan and or MRI findings were obtained. The specimens were received intra-operatively. The tissues were labelled and numbered. The smears were prepared by lightly touching 1-2 mm tumour tissue on the glass slide. Simultaneously, two crush preparations were also made by cutting small representative tissue with scalpel blade. The tissue was placed on a glass slide and sufficient pressure applied to make smears. Second slide was placed longitudinally on the first, light pressure was applied to flatten the tissue and slides were drawn apart in opposite directions.

Both touch and crush smears were fixed without delay in 95% ethanol and stained with rapid haematoxylin and eosin stains. Remaining tissue was fixed in 10% buffered formalin for permanent histopathological sections.

Consultant pathologist was informed prior to the craniotomy. As soon as the slides were ready, these were studied under light microscope. Relevant clinical history and data were taken into full consideration while concluding diagnosis. Usually, neurosurgeons were informed about the diagnosis within 15 minutes.

Considering histopathology as final diagnosis, the sensitivity, specificity, positive and negative predictive values were calculated.

Table 1 and 2 show the cytological criteria used for grading on touch preparation of astrocytomas.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Low grade</th>
<th>High grade</th>
<th>GBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisonucleosis</td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
</tr>
<tr>
<td>Poikilocytes</td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
</tr>
<tr>
<td>Nuclear membrane breaks</td>
<td>Absent</td>
<td>Present</td>
<td>Frequent</td>
</tr>
<tr>
<td>Chromatin texture</td>
<td>Smooth</td>
<td>Granular</td>
<td>Irregular</td>
</tr>
</tbody>
</table>

*Cellularity: + <2 clusters of astrocytes, ++: 2-5 clusters of cells, +++: >5 clusters*

For correspondence
Dr. Humaira Nasir,
Department of Pathology,
Pakistan Institute of Medical Sciences, Islamabad.
Results

During the period of study, astrocytoma was diagnosed in 40 cases. The age ranged between three months and sixty years. Astrocytomas were graded into low-grade, high-grade and glioblastoma multiforme from January 1999 to September 2001.

The break-up of 40 cases of astrocytoma, on the basis of criteria detailed in tables 1 & 2, into low grade, high grade & glioblastoma multiforme, is compared with final histological diagnosis in Table 3.
On cytological examination (Figs. 1-4), the low-grade astrocytoma appeared moderately cellular, with some degree of pleomorphism on a finely fibrillar background. The nuclear shape, contour and chromatin content closely resembled those of normal/reactive astrocytes. Nuclei tended to be round to oval, and often had a fine, slightly coarser than normal but evenly distributed chromatin. Nucleoli were small or inconspicuous. The cell borders appeared indistinct and naked nuclei were frequent.

The high-grade astrocytomas were characterized by a greater degree of nuclear pleomorphism, binucleation or multinucleation, eccentrically placed nuclei, coarser chromatin and frequently prominent nucleoli. They also displayed a greater cellularity on a denser and fibrillar background.

The glioblastoma multiforme revealed marked nuclear atypia. Additionally there were vascular & endothelial proliferation and necrosis.

On histological examination, one case diagnosed cytologically as reactive gliosis turned out to be low-grade astrocytoma. Seventeen out of 19 cases were confirmed histologically as low grade, one case was of reactive gliosis, and one was mixed glioma (oligoastrocytoma). Fifteen cases of high-grade astrocytoma were correctly graded on touch preparation. Five cases of glioblastoma multiforme were also graded correctly on touch cytology.

The touch and crush cytology, in the intra-operative diagnosis of astrocytomas, was found to have a sensitivity of 98.4% and a specificity of 90%.

**Table 3: Astrocytoma as Diagnosed on Touch and Histopathology**

<table>
<thead>
<tr>
<th>Method Used</th>
<th>Reactive Gliosis</th>
<th>Low grade</th>
<th>High grade</th>
<th>GBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>1</td>
<td>19</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Histopathology</td>
<td>1</td>
<td>17</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Total Cases</td>
<td>1</td>
<td>17</td>
<td>15</td>
<td>5</td>
</tr>
</tbody>
</table>

This study included 40 cases of astrocytoma. In 19 of them, astrocytoma was low grade, and in 15 cases it was high grade. In the remaining 5 cases, a diagnosis of glioblastoma multiforme was made. One case was diagnosed as having reactive gliosis. There was no inconsistency in 17 out 19 cases of low-grade glioma on examination of histological sections. One case was found out to be having reactive gliosis and the other one had mixed glioma of low-grade nature. The oligodendroglial component was not recognized on cytology. None of fifteen cases of high grade and five cases of glioblastoma multiforme showed inconsistency on permanent histopathological examination. On the other hand, one case of reactive gliosis turned out to be low-grade astrocytoma on permanent sections.

It has been previously mentioned that the diagnosis of low-grade astrocytoma, especially grade-1 fibrillary type, may be difficult to diagnose cytologically as well as histologically. The differentiation of normal, reactive and neoplastic astrocytes depends on identification of cytologic abnormalities. The astrocytes of normal brain appear small and do not contain recognizable cell processes. The astrocytes of reactive gliosis show cell processes and mild degree of cytologic abnormality that overlaps with slight degree of abnormality seen in low grade astrocytoma. It was therefore not surprising to have difficulty in two cases of low-grade astrocytoma and one case of reactive gliosis. Low-grade astrocytomas show mild hypercellularity and slight cytologic atypia and pleomorphism. The biopsy from the central part of the tumour may be more representative. A biopsy taken from a peripheral area can be misdiagnosed cytologically due to sampling error.

We observed one case of mixed low-grade oligo-astrocytoma, which was misdiagnosed as low-grade astrocytoma due to focal nature of the lesion.
Similar observations have been reported in a previous study comprising of 217 cases. In this study, intra-operative smear technique was assessed in diagnosis of reactive astrocytes and low-grade neoplastic astrocytes. The frequent diagnostic errors were under-grading of gliomas (20 cases), incorrect assessment of their histogenesis (10 cases), diagnosis of low-grade astrocytoma in case of reactive gliosis (3 cases), and diagnosis of glioblastoma multiforme instead of metastatic carcinoma (2 cases).

In another study, Robbins et al evaluated 100 stereotactically biopsied intra-cerebral lesions along with touch preparation. They observed two broad areas of diagnostic difficulty in interpretation of touch cytology, i.e., tumour typing & grading, and distinction between reactive and neoplastic astrocytic proliferation.

The differentiation of low-grade from high-grade astrocytoma depends on established limits of cellularity and cytologic atypia. The presence of more than slight hypercellularity and cytologic atypia along with mitotic figures likewise indicates that the neoplasm is a high-grade astrocytoma. It is recognized by the presence of cohesive syncitial groups of endothelial cells arranged either as clumps or in a linear fashion and interspersed with the neoplastic astrocytes. The spindle cells of these abnormal vessels are arranged parallel to each other, an important point of distinction from normal muscular arterioles that may be present. The endothelial cell proliferation and necrosis in the background of a hypercellular smear lead to the diagnosis of glioblastoma multiforme. Necrosis appears as clumps of acellular eosinophilic debris.

Morphometric assisted grading of astrocytoma on cytological smears is now being practised using automated equipment. In this technique, quantitative features pertaining to entire nucleus, nucleolus along with optical density and quantitative chromatin pattern description are taken into account. High-grade astrocytomas usually have a different C.T. scan appearance from low-grade astrocytoma. They are more irregular with areas of different C.T. density, and usually show enhancement with contrast. When a biopsy specimen from a lesion has feature of high grade astrocytoma on C.T., but features of low-grade astrocytoma on cytology, we advise neurosurgeons to take additional biopsies from other locations to avoid sampling errors. A diagnosis of low-grade astrocytoma is made preferably when both smear and C.T scan appearances are consistent with this diagnosis. This avoids mistaken diagnosis due to sampling error.

**Conclusion**

It was concluded that:

1. The touch and crush cytology are useful in the intra-operative diagnosis of astrocytomas with a sensitivity of 98.4% and a specificity of 90%.
2. Brain tissue because of a high lipid content and soft nature frequently develops serious freeze artefacts; therefore, touch & crush cytology provide much crisper and artefacts free cells on small tissue samples.
3. As astrocytomas vary in grade in different areas and as only limited number of tissue holders are available in cryostats, the cytological examination permits much wider sampling and examination with much less effort and cost.
4. The study is of special value in countries like Pakistan where cryostat facility is not available at many centres. Moreover, cytology provides more crisp cytologic detail than frozen sections, and most of freezing artefacts in brain are avoided.
5. The cytological techniques being simple, cost-effective, fast and accurate are quite useful in the intra-operative craniotomy brain biopsies.

**Acknowledgement**

The authors acknowledge the great help of Prof. Khalid Hassan in reviewing and editing the manuscript.

**References**


